Development of macrocyclic *β*-strand calpain cysteine protease inhibitors

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Contents

Abstract

The work in this thesis reports studies directed to developing a calpain cysteine protease inhibitor that could be of value in slowing cataract development in humans. The work focuses on the development of macrocyclic compounds which can have advantages over acyclic compounds due to their resistance to proteolytic hydrolysis, improved selectivity, bioavailability and membrane permeability. A review of X-ray crystal structures of natural and synthetic calpain inhibitors complexed with the cysteine protease calpain show the inhibitors generally bind in the enzyme active site in an extended β-strand conformation.

The calpain inhibitor **SJA-6017** has been identified as a suitable lead compound. The importance of the *para*-fluoro group in **SJA-6017** has been investigated. Modifications have been made to constrain this basic structure within a macrocycle and restrict the peptide chain as a β-strand conformation. Macrocycle **CAT811** is a potent calpain 1 and 2 inhibitor and shows promise in slowing the progression of cortical cataract in trials with sheep having a hereditary propensity towards the development of cataract.

In this thesis I report studies directed to improve the yield of the key RCM macrocyclisation step in the synthesis of aldehyde **CAT811** and of three ester analogues (**2.1**, **2.3** and **2.4**).

I also report the development of a more commercial route to **CAT811** not involving RCM but using intramolecular nucleophilic cyclisation.

This intramolecular nucleophilic cyclisation strategy was attempted for the preparation of a histidine containing macrocyclic ester (**4.1a**) but was unsuccessful. An alternate strategy involving intramolecular lactamization proved successful for the synthesis of histidine-based macrocyclic esters (**4.1a**-**4.3a**). Reduction to the corresponding alcohols

(**4.1b**-**4.3b**) was successful and oxidation of (**4.1b** and **4.3b**) afforded the corresponding aldehydes (**4.1c** and **4.3c**) for biological assay against ovine calpain 2.

Aldehyde **4.3c** has an IC_{50} of 1 μM and the corresponding alcohol **4.3b** shows no activity (IC_{50} > 50 μM) consistent with the modelling which indicated that these two compounds did not adopt a *β*-strand conformation in the docking studies. Aldehyde **4.1c**, on the other hand, shows significant inhibitory activity with an IC_{50} of 238 nM but as expected the corresponding alcohol **4.1b** shows little activity (IC_{50} = 29 μM). Modelling studies showed that both the aldehyde **4.1c** and the alcohol **4.1b** on docking can form a *β*-strand with appropriate H-bonding interactions. The aldehyde is more active than the alcohol due to the reactivity of the aldehyde warhead allowing for the reversible formation of a hemiacetal. A similar difference in reactivity is observed for **CAT811** (30 nM) and its alcohol analogue (700 nM).

These results demonstrate the value of molecular modelling as a screening mechanism before unproductive synthetic work is considered.

Work in this thesis has been published in the following papers:

Abell, A. D.; Alexander, N. A.; Aitken, S. G.; Chen, H.; Coxon, J. M.; Jones, M. A.; McNabb, S. B.; Muscroft-Taylor, A. *J. Org. Chem.*, **2009**, *74*, 4354–4356.

Jones, M. A.; Coxon, J. M.; McNabb, S. B.; Mehrtens, J. M.; Alexander, N. A.; Jones, S.; Chen, H.; Buisan, C.; Abell, A. D. *Aust. J. Chem.*, **2009**, *62*, 671–675.

Zaman, S.; Chen, H.; Abell, A. D. *Tetrahedron Lett.*, **2011**, *52*, 878–880.

Abbreviations

1,1,2-TCE	1,1,2-Trichloroethane
Boc	*tert*-Butoxycarbonyl
Cbz	Benzyloxycarbonyl
COSY	Correlation spectroscopy
DCM	Dichloromethane
d	Doublet (in NMR)
dd	Doublet of doublets (in NMR)
ddd	Doublet of doublets of doublets (in NMR)
dt	Doublets of triplets (in NMR)
DIPEA	*N*,*N*-Diisopropylethylamine
DMAP	4-(Dimethylamino)pyridine
DMF	*N*,*N*- Dimethylformamide
DMSO	Dimethyl sulfoxide
EtOAc	Ethyl acetate
EDCI	1-[3-(Dimethylamino)propyl]-3-carbodiimide hydrochloride
equiv	Equivalent(s)
ES	Electrospray ionization
Grubbs' second generation catalyst	Benzylidene[1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene]dichloro(tricyclohexylphosphine)ruthenium.
h(s)	Hour(s)
HATU	*N*,*N*,*N*',*N*'-Tetramethyl-*O*-(7-azabenzotriazol-1-yl)uranium hexafluorophosphate
HCl(aq)	Hydrochloric acid
HMBC	Heteronuclear multiple bond correlation (in NMR)
HOAt	1-Hydroxyazabenzotriazole
HRMS	High resolution mass spectroscopy
Hz	Hertz (in NMR)
HSQC	Heteronuclear single quantum correlation (in NMR)

J	Coupling constant
$LiAlH_4$	Lithium aluminium hydride
$LiBH_4$	Lithium borohydride
LRMS	Low resolution mass spectroscopy
m	Multiplet (in NMR)
Mp	Melting point
MeOH	Methanol
$MgSO_4$	Magnesium sulfate
$NaHCO_3$	Sodium bicarbonate
NaOH	Sodium hydroxide
NMR	Nuclear magnetic resonance
Pet ether	Petroleum ether
Pd/C	Palladium on carbon catalyst
ppm	Parts per million
RCM	Ring closing metathesis
rt	Room temperature
s	Singlet (in NMR)
SARs	Structure activity relationships
SO_3.Pyr	Sulfur trioxide pyridine complex
t	Triplet (in NMR)
TFA	Trifluoroactetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
δ	Chemical shift (in NMR)

Acknowledgements

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Chapter 1: Introduction

Calpains are Ca^{2+}-dependent cysteine proteases that selectively cleave specific proteins in response to calcium signals.[1] There are at least fifteen different calpain isoforms that have been identified from a variety of organisms and mammals and each isoform is encoded by an independent gene (Table 1.1).[2] Some of these isoforms are expressed ubiquitously (calpains 1, 2, 4, 5, 7, 10, 12 and 13), while others are tissue-specific (calpains 3, 6, 8, 9 and 11). For example, calpain 3 is a skeletal muscle-specific enzyme[3] and calpain 8 is stomach-specific.[4] Calpain 9 is tissue-specific with an express pattern restricted to the digestive track.[5] Calpain 12 is highly expressed in mouse hair follicles.[6] Calpains 11 and 13 are mainly detected in testes.[7]

Calpains are involved in the regulation of many biological processes and their physiological functions define important pathological roles in human diseases (Table 1.1). For example, deregulation of calpain activity is involved in neurological disorders, such as Alzheimer's, Huntington's and Parkinson's diseases and in multiple sclerosis.[8,9] An increased Ca^{2+} level in muscles leads to calpain over activation and is associated with muscular dystrophy.[10] The proteolytic action of calpain 2 on lens crystallin proteins is thought to be a cause of cataracts in humans.[11] The ageing process or an insult to the eye can result in elevated levels of Ca^{2+} in the lens tissue which leads to calpain over activation with associated deregulative proteolysis of soluble crystallins and cataract formation. This process is the topic of this thesis.

Table 1.1. The calpain family[12]

Calpain	Other names	Chromosome	Expression	Isoforms	Crystal structure	KO mouse phenotype	Known targets	Diseases
CAPN1	μ-calpain	11q13.1	Ubiquitous	-	Catalytic domain	Hematopoietic homeostasis alterations (MGI:88263)[a]	Multiples[b]	Huntington's disease, cataracts, stroke, muscular dystrophy, traumatic brain injury, spinal cord injury, alzheimer, cancer, multiple sclerosis, Lou Gehrig's disease, osteopenia
CAPN2	m-calpain	1q42.11	Ubiquitous	-	Complete	-	Multiples[c]	Cataracts, muscular dystrophy, stroke, spinal cord injury, traumatic brain injury, Alzheimer, Parkinson, atherosclerosis, multiple sclerosis, Lou Gehrig's disease, cancer, psoriasis
CAPN3	p94, nCL-1	15q15.1	Skeletal muscle, lens, retina	Yes (8)	-	Muscular dystrophy. Transmission distortion (MGI:107437)	Calpastatin	Limb girdle muscular dystrophy 2A (LGMD2A), cataracts
CAPN4	CAPNS1, CSS1	19q13.12	Ubiquitous	-	Domain VI	Embryonic lethality. Cardiovascular and erythropoyesis alterations (MGI:88266)	-	-
CAPN5	hTRA-3, nCL-3	11q13.5	Ubiquitous (high in colon, small intestine and testis)	-	-	Usually normal, occasionally reduced body weight (one allele). Embryonic lethality (another allele) (MGI:1100859)	Huntingtin	Huntington's disease polycystic ovarian syndrome, metabolic syndrome
CAPN6	CAPNX	Xq23	Placenta	-	-	-	-	-
CAPN7	PalBH	3p25.1	Ubiquitous	-	-	Postnatal lethality Decreased body weight (MGI:1338030)	Huntingtin	Huntington's disease
CAPN8	nCL-2	1q32-q41	Stomach mucosa	Yes (2)	-	Unknown (MGI:2181366)	-	-
CAPN9	nCL-4	1q42.2	Digestive track	Yes (2)	Catalytic domain	-	-	Gastric cancer
CAPN10	CAPN8	2q37.3	Ubiquitous and tissue-specific	Yes (8)	-	-	Huntingtin Crystallin(?)	Huntington's disease, cataracts (?), diabetes mellitus, atherosclerosis, metabolic syndrome
CAPN11	-	6p21.1	Testis	-	-	-	-	-
CAPN12	-	19q13.2	Ubiquitous (high in hair follicle)	-	-	-	-	Alzheimer (?)
CAPN13	-	2p23.1	Testis and lung	-	-	-	-	-
CAPN14	-	2p23.1	Ubiquitous	Yes (2)	-	-	-	-
CAPN15	Sol H	16p13.3	Ubiquitous	-	-	-	-	CATM (hereditary cataract with microphthalmia) (?)
CAPNS2	CAPN14 CSS2	16q12.2	Lens	-	Domain VI	-	-	-

[a] MGI: Mouse Genome Informatics database.
[b] Filamin, Talin, Pyk2, FAK, GFA, PKCα, Spectrin, Cadherin, Tau, Bcl-2 family, Caspase 12, Fodrin, MAP-2, Huntingtin, IκBα, Integrin, IGFBPs, ICA512, AQP2, XIAP, RhoA, Preselin, PTP-1B, Cortactin, Caldesmon, Calponin.
[c] Crystallin, Filamin A, Tau, Tali, Paxilin, IκBα, Fodrin, Pyk2, PKCα, δ, GAP-43, FAK, pp60src, p53, Cyclin E, Preselinin, Cortactin, Caldesmon, Calponin, Catenin, RNase L, CaMK.

Two calpains isoforms, calpain 1 (μ-calpain) and calpain 2 (*m*-calpain), have been extensively studied.[1] Calpain 1 and 2 each consist of an 80kDa catalytic subunit (the large subunit), containing four domains (DI-DIV), and a 28kDa regulatory subunit (the small subunit), containing two domains (DV and DVI). Domain I is a short helix. Domain II is a ubiquitous catalytic domain in which calpain activity is located. Domain III is a C2-like domain that is involved in membrane targeting. Domains IV and VI are calcium bonding segments with five EF hand motifs and these two domains are linked through the pairing of their fifth- EF hand (Figure 1.1).

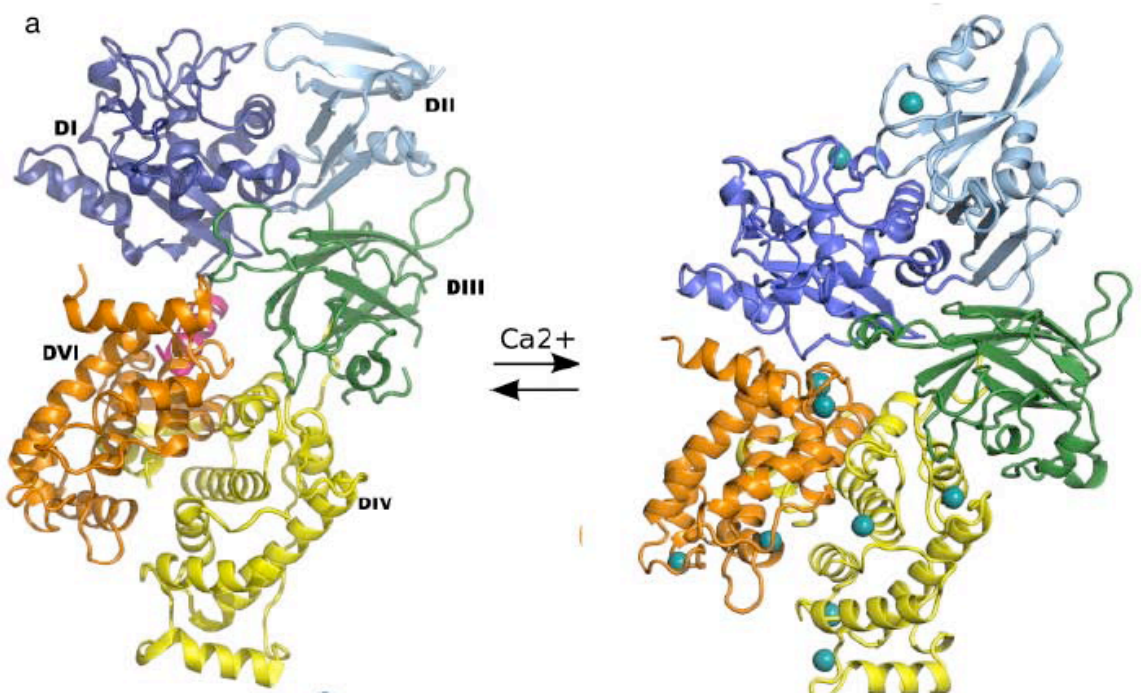


Figure 1.1: A comparison of calcium-free (left) and calcium-bound (right) calpain structures (Ca^{2+} ions are shown as teal spheres).[13]

Calpain 1 and calpain 2 require micromolar and millimolar concentrations of calcium ions (Ca^{2+}) respectively for *in vitro* activation. The binding of calcium ions induces a change in the relative positions of the domains of calpain, leading to its active conformation. The protease core contains two calcium ions, one in each of DI and DII

domains, and these are responsible for the cooperative assembly of the catalytic cleft. Four calcium ions are bound in both EF-hands of DIV and DVI and result in a shift in the EF hands, exposing a hydrophobic site allowing for substrate or inhibitor binding.

1.1: Calpastatin, an endogenous calpain inhibitor

1.1.1: Overview of calpastatin

The activity of calpains 1 and 2 is tightly controlled by the endogenous inhibitor calpastatin, which exhibits IC_{50} values of 0.50-1.57 nM and 0.83-1.34 nM respectively. Calpastatin, a 120kDa protein widely distributed in most mammal tissues, comprises four inhibitory domains with each domain containing subdomains (A, B and C), and an *N*-terminal region L (Figure 1.2).[14] All four repeat units have similar inhibitory activity against calpains 1 and 2, but domain L is devoid of such activity.

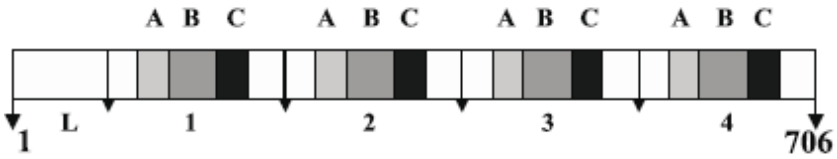


Figure 1.2. Repeat domain structure of human calpastatin. Regions A (20 residues), B (26 residues) and C (20 residues) are highly conserved among the four internally repeated domains 1–4.

There are difficulties associated with the co-crystallization of the native calpain in the presence of calcium ions because the activated calpain hydrolyzes itself into inactive fragments.[15] An inactive mutant of calpain 2 (C105S) has been co-crystallized with calpastatin's inhibitory domain IV in the presence of calcium (Figure 1.3). Calpastatin domain IV (black) binds as an extended polypeptide over the surface of calpain, making contact with each domain of enzyme. The regions A and C of calpastatin domain IV bind to the hydrophobic calpain domain DIV and DVI and fold as a α-helix respectively. The

B subdomain of calpastatin binds to the calpain domains I and II in an extended orientation and blocks the active site of calpain.

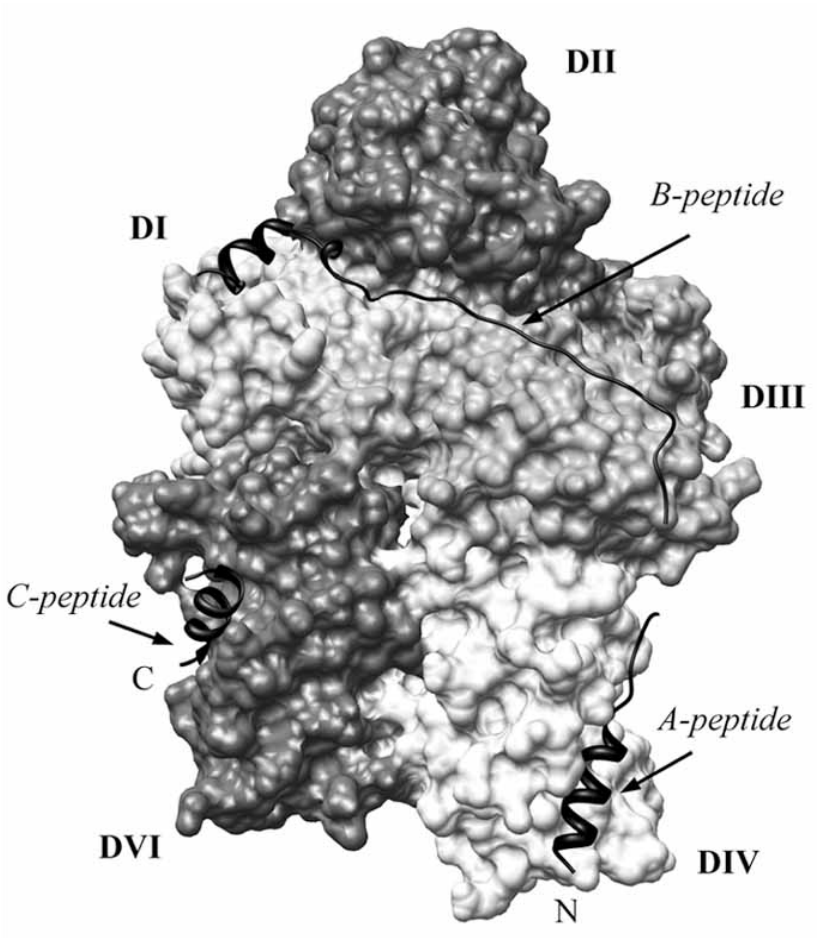


Figure 1.3. Complex between calpastatin domain IV and Ca^{2+}-bound calpain 2.[1,13]

1.1.2: Calpain-calpastatin interactions proximate to the active site of calpain

Calpain presents a reaction surface to which its substrate peptide binds as defined by standard nomenclature developed by Berger and Schechter (Figure 1.4).[16] Subsites that bind residues on the *N* terminal side of the substrate (or non-primed sites) are numbered as S1 – Sn and those toward the *C* terminus (primed sites) S1'– Sn', beginning from the

sites on each side of the scissile bond. The corresponding substrate residues are numbered P1–Pn and P1'–Pn' respectively as shown in Figure 1.4.

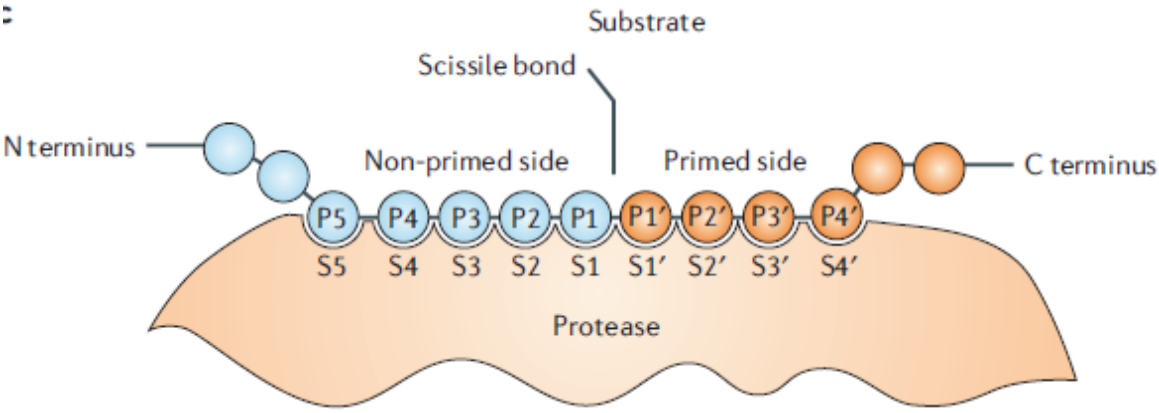


Figure 1.4. Schechter and Berger nomenclature for substrate residues bound with the active site of protease as reported by Turk. [17]

The active site of calpains consists of a catalytic triad of amino acids, namely cysteine, histidine and asparagine that directly participate in the hydrolysis of the peptide substrate. The nucleophilic thiol (SH) of cysteine (for calpain 2, Cys 105) is positioned to react with a substrate to form an intermediate with a low-energy transition state thereby facilitating an increase in the rate of hydrolysis. The imidazole side chain of the histidine (for calpain 2, His262) functions by accepting and donating a proton during the reaction. The aspartate side chain (for calpain 2, Asn286) interacts by way of a hydrogen bond to orient the histidine appropriately to remove the acidic proton from the thiol group of cysteine (see Figure 1.5). This deprotonation is the first step in peptide bond hydrolysis. The next step involves nucleophilic attack by the activated cysteine's anionic sulfur on the substrate carbonyl carbon to form a thioester intermediate. Donation of a proton by histidine to the peptide amide nitrogen forms a protonated amine which facilitates amide bond cleavage to release a fragment of the substrate having an amine terminus. Deprotonation of a water molecule by the histidine base is followed by nucleophilic attack at the carbonyl carbon to generate a tetrahedral intermediate which collapses to a carboxylic acid and release of free enzyme.

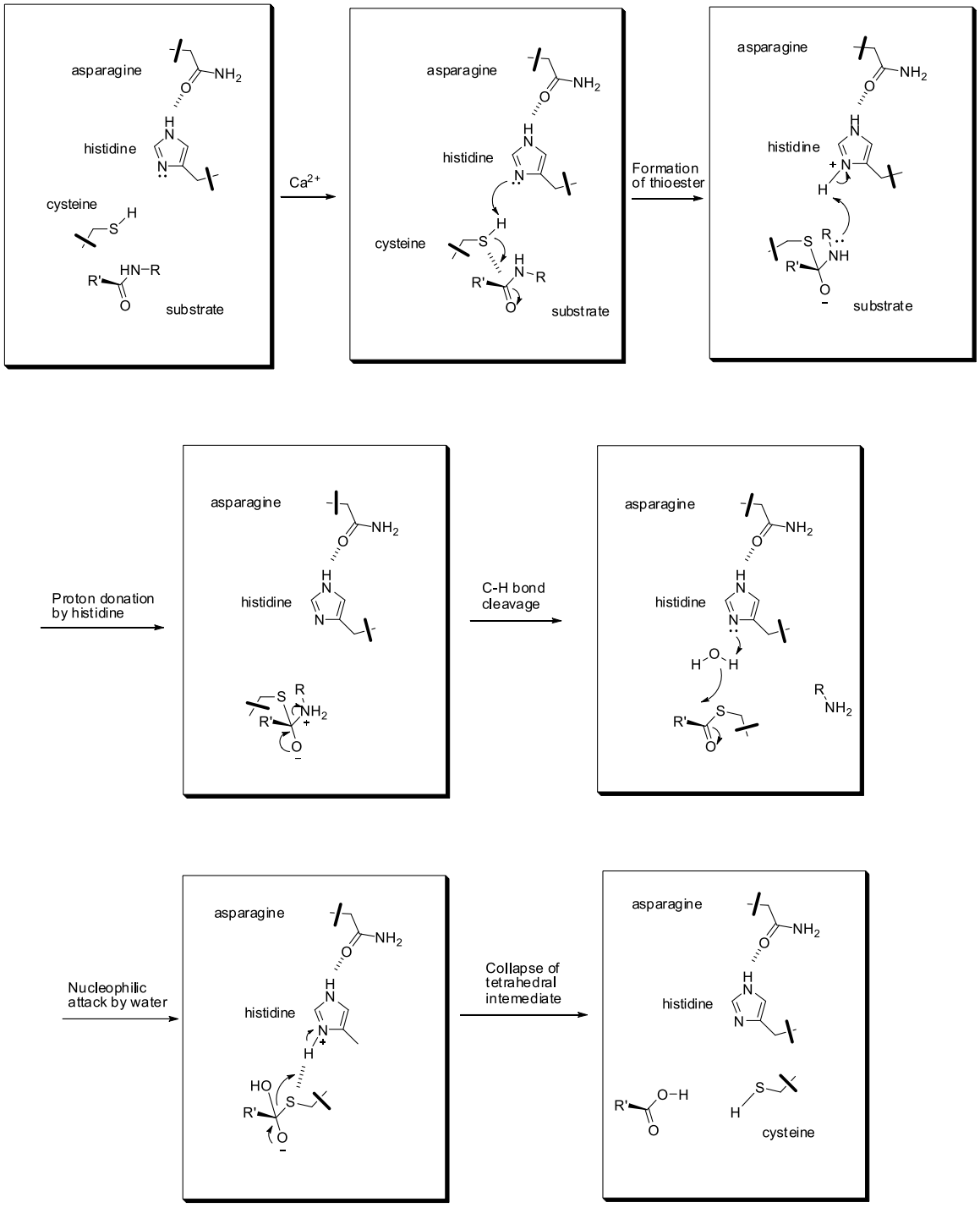
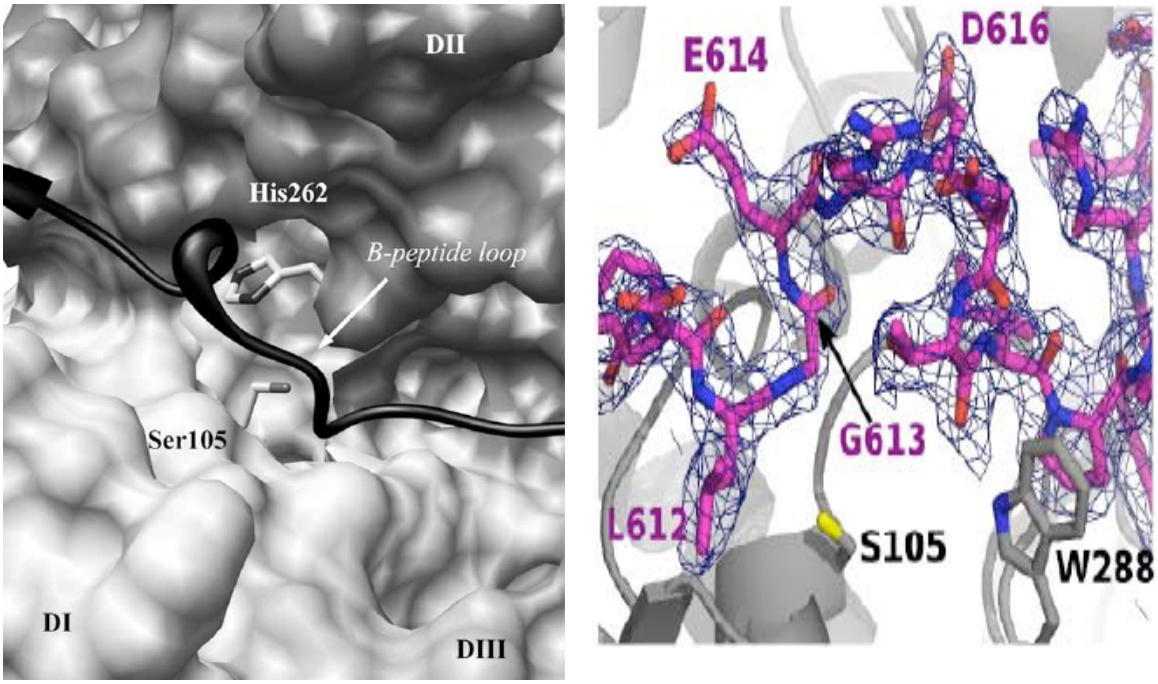


Figure 1.5. The proposed mechanism of action of cysteine protease.[18]

The binding of calpastatin to the active site Cys(Ser)105 of a mutant of calpain 2 disrupts this mechanism and is shown in Figure 1.6a. This crystal structure shows the B subdomain of the calpastatin binds with calpain domain DI-DIII and thereby obstructs the active site.[1] The region on the *N*-terminal side (domain L) of the inhibitor forms hydrophobic and electrostatic interactions with residues in a shallow trough in calpain's domain III. By contrast, the region C-terminal to the loop of subdomain B forms a two-turn amphipathic α- helix that binds to domain I (also see Figure 1.3).



(a) (b)

Figure 1.6. a) Central region of subdomain B of calpastatin's domain IV (black) looping around the active site cysteine (serine) of calpain 2. b) Close-up views of calpastatin at the active site.

The close-up view of calpastatin binding to the active site of calpain 2 shows that Leu612 occupies the hydrophobic S2 subsite (Figure 1.6b). A type II *β*-turn conformation in calpastatin twists the P1 Gly613 of calpastatin away from the active site and holds the potentially scissile amide bond between P1 Gly613 and P1' E614 of calpastatin about 2 Å

removed from the cysteine thiol, thereby preventing hydrolysis as a result of the looping of calpastatin away from the active site cysteine.[19]

The 27-residue peptide Ac-DPMSSTYIEELGKREVTIPPKYRELLA-NH_2, consisting of a repeat sequence of domain I of human calpastatin, has been synthesized and tested for inhibitory potential.[20] This polypeptide is a highly potent and selective inhibitor of calpain 1 and 2 in a nanomolar range, but it does not however inhibit either papain or trypsin.[21]

1.2: A survey of other natural and selected synthetic calpain inhibitors

A number of naturally occurring calpain inhibitors have been reported as shown in Figure 1.7.[22] For example Leupeptin (Ac-Leu-Leu-Arg-H) and Strepin P-1 (N-i-valeryl-Tyr-Val-Arg-H), isolated from *Streptomyces* species, inactivate calpain by reacting reversibly with the active site thiol of the enzyme. The peptidylepoxysuccinate inhibitor **E-64**, isolated from *Aspergillus japonicas,* acts as a non-selective calpain inhibitor with the epoxide reacting with an active site thiolate group to form a covalent bond. Inhibitor **SC488**, isolated from *Streptomyces griseus*, has an IC_{50} value of 1.2 μM against calpain.[23]

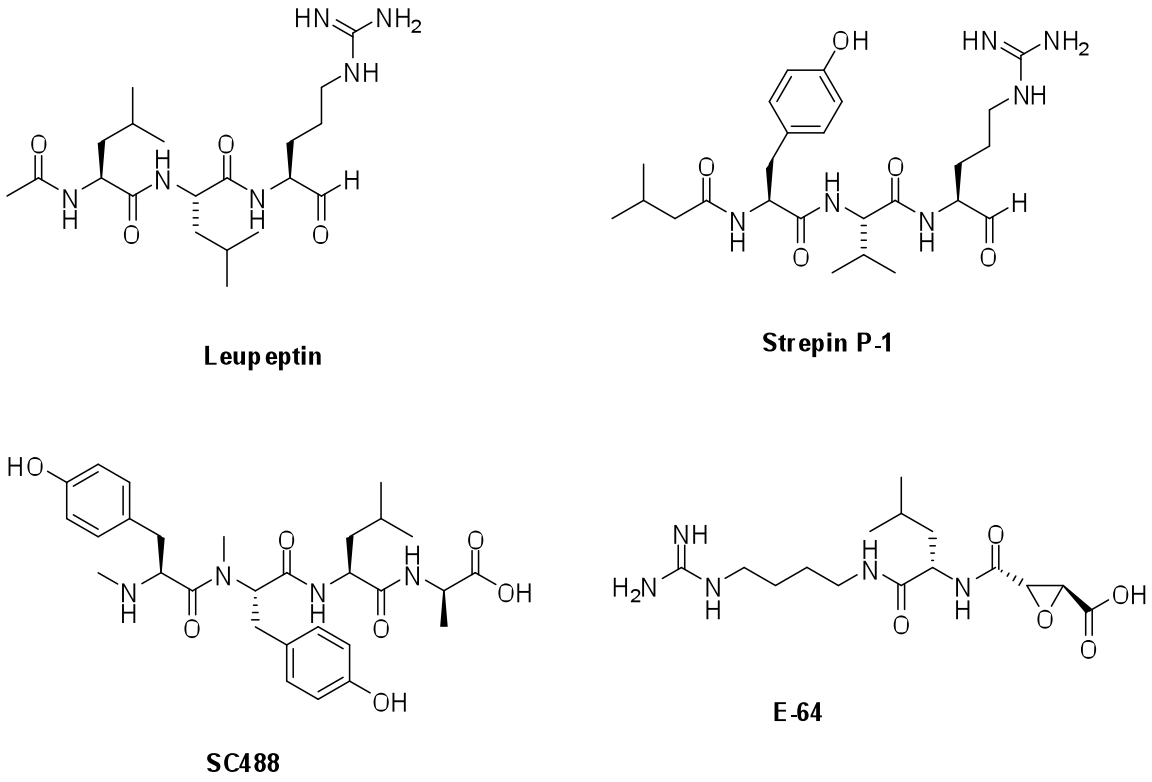


Figure 1.7. Representative calpain inhibitors derived from natural sources.

These natural calpain inhibitors tend to have undesirable properties; for example a lack of selectivity and poor membrane cell permeability, absorption, and metabolic stability. A number of synthetic peptide- and nonpeptide-based calpain inhibitors have also been reported.[24] These inhibitors have the scissile amide bond of a calpain substrate replaced with a functional group (warhead) that reacts with the active site thiolate cysteine either reversibly or irreversibly. Such inhibitors are classified into three groups according to the nature of the warhead. These include epoxysuccinate derivatives, aldehydes, aldehyde prodrugs (hemiacetals), and α-keto carbonyl compounds.

While the epoxysuccinate derivative **E-64** (Figure 1.8) functions as a cysteine protease inhibitor, it has the disadvantage that it is not permeable to cells because the ionizable carboxylic acid and guanidinium functional groups prevent it crossing cell membranes. The cellular penetration of **E-64** has been improved by esterification of its carboxylic

acid and replacing the guanidinium group with an alkyl group as in **E-64D**.[25] This inhibitor has been shown to provide significant neuroprotection after spinal cord injury in rat.[26] The ester group in **E-64D** undergoes *in vivo* hydrolysis to give the active inhibitor **E-64C**. Both cell permeability and inhibitory activity of **E-64** are improved by incorporation of a lipophilic *N*-terminal capping such as benzyloxycarbonyl (**Ep-460**).

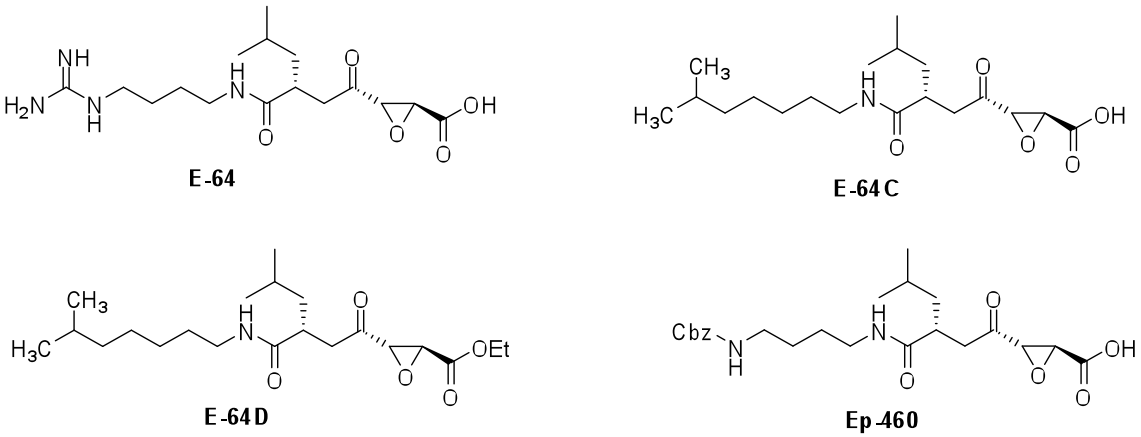


Figure 1.8. Examples of irreversible epoxysuccinate calpain inhibitors.

Peptide aldehydes represent the largest class of known calpain inhibitors. These inhibitors function by reversibly reacting with the active site cysteine thiol. For example, Leupeptin (Figure 1.7) inactivates both calpain 1 and calpain 2 with IC_{50} values of 0.27 μM and 0.38 μM, respectively. However its poor cell permeability results in this compound being unattractive as an *in vivo* agent. Modification of leupeptin with a NH_2-terminal protected by a benzyloxycarbonyl group and removal of the guanidinium group results in a derivative [Calpeptin, Z-Leu-Nle-H] with improved cell permeability (Figure 1.9).[27]

Peptide aldehydes **MDL-28170**, **SJA-6017**, **ALLN** and **ALLM** (Figure 1.9) all function as effective inhibitors of both calpain 1 and calpain 2. Administration of **MDL-28170** reduces the neuronal loss and improved locomotive functions in a rat model of Parkinson's disease.[28] The dipeptide aldehyde **SJA-6017** is a potent calpain 2 inhibitor that is effective against selenite-induced cataract in rat.[29] The tripeptide aldehyde **ALLN**

has been used to reduce neuronal damage in a rat model of focal ischaemia and as a chemotherapeutic agent to overcome acquired resistance in cancer therapy.[30] Calpain inhibitor **ALLM** is reported to provide effective neuroprotection *in vitro* and *in vivo* models of spinal cord injury and cerebral ischemia.[31]

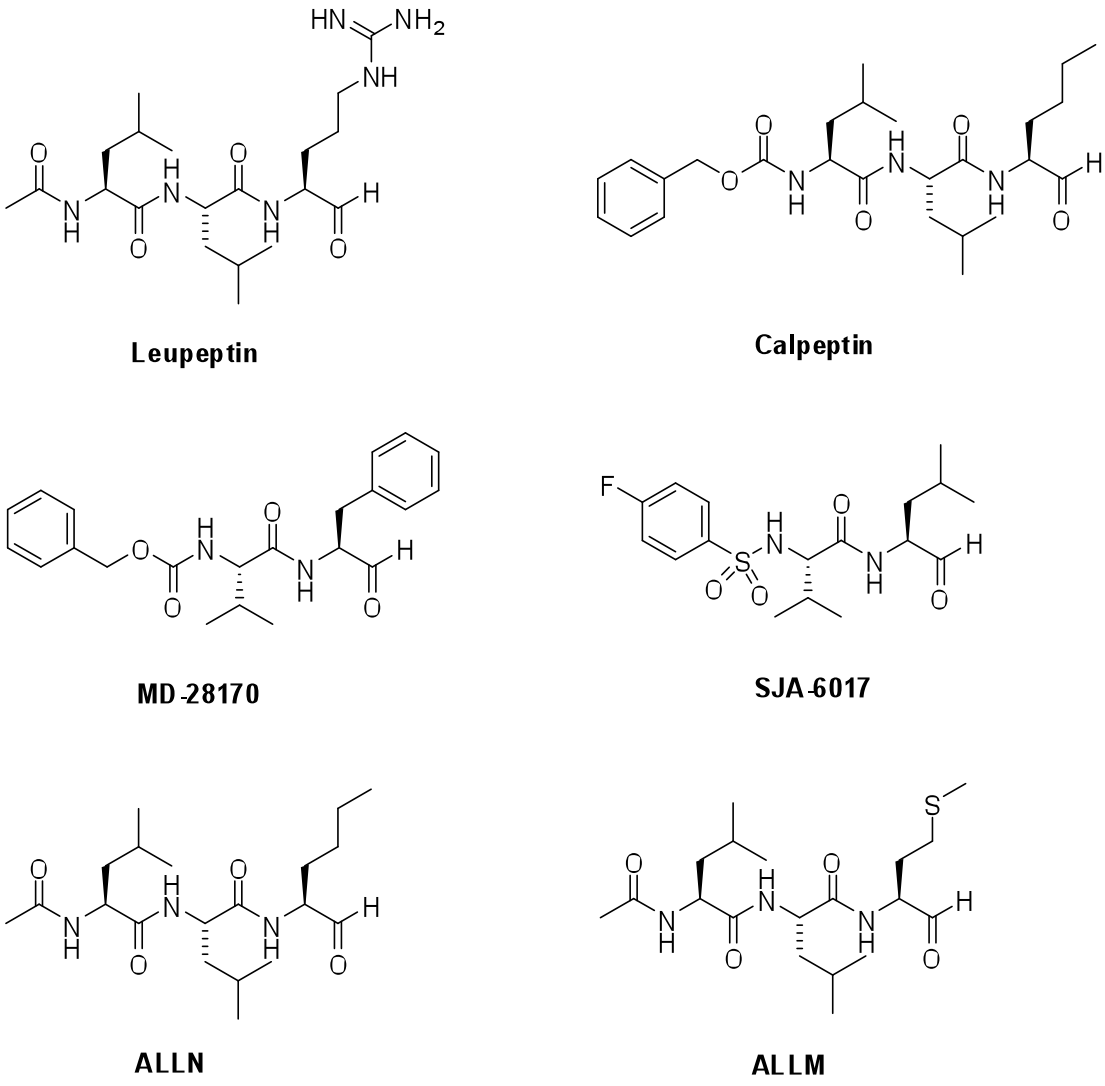


Figure 1.9. Examples of peptide aldehydes as reversible calpain inhibitors.

1.3: Evaluation of calpain inhibitors for the treatment of cataract

Cataract is a disease of the eye. It results in the lens becoming opaque with a decrease in the quality of vision and ultimately blindness. The lens of the human eye is a highly specialized non-vascular tissue that focuses an image onto the retina of the eye, triggering electrical impulses that are interpreted by the brain as vision (Figure 1.10).[32]

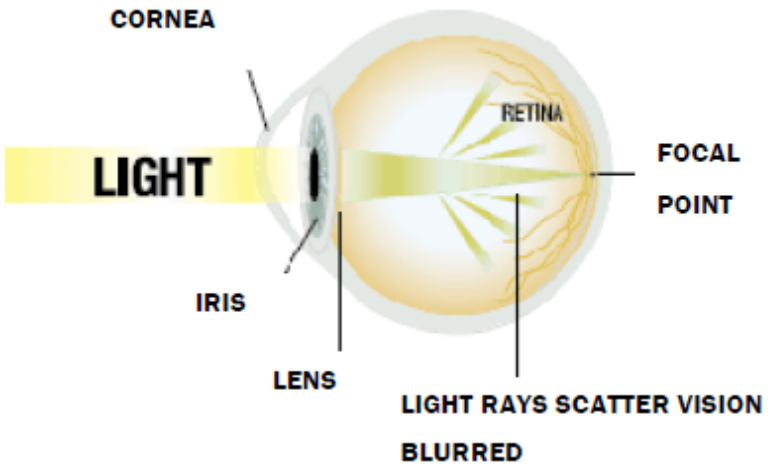


Figure 1.10. The difference in focusing of lighting between normal and lens with a cataract.

There is increasing evidence that the over activation of calpain is implicated in some forms of cortical cataract.[33,34] Calpain 2 is the major calpain isoform found in the epithelial cell of the human eye lens and is thought to be important in cortical cataract.[35] Cataract formation results from a variety of insults such as genotype, selenite and damage from ultraviolet light (Figure 1.11). Recent work has shown that as the human lens ages, the intensity of light scattered backwards out of the pupil can often increase exponentially.[36]

It has been proposed that the ageing process results in elevated levels of Ca^{2+} in lens tissue and hence calpain over activation with associated deregulated proteolysis of

soluble crystallins giving rise to precipitation and aggregation. This compromises the function of crystallins in maintaining lens transparency and results in the reduced lens performance associated with cataract (Figure 1.11).[37,38,39] Development of calpain inhibitors that could slow cataract progression is considered of scientific and commercial interest.

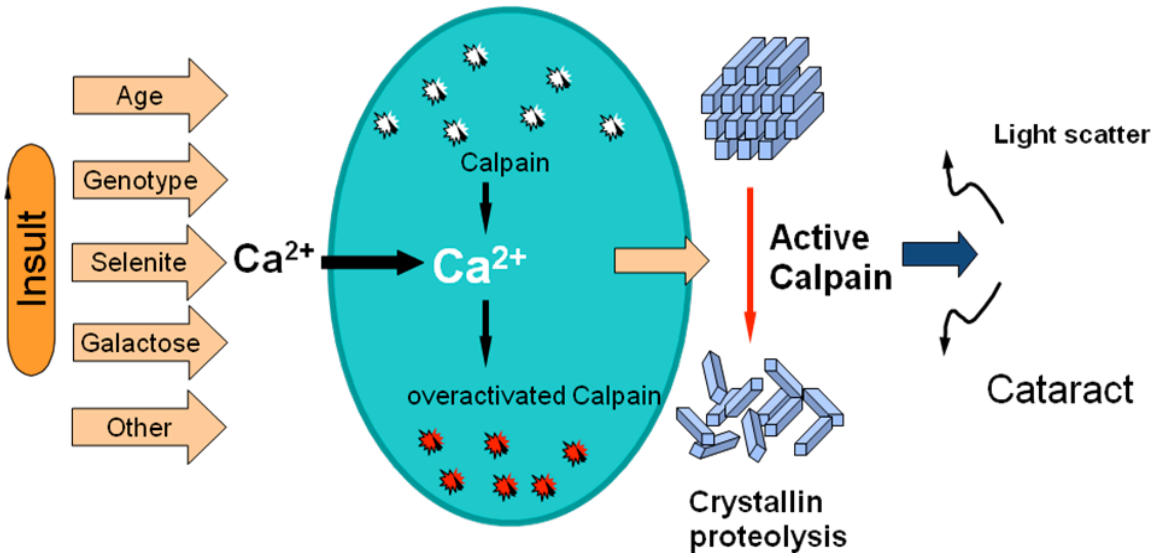


Figure 1.11. The origin of cataract.

Currently the only treatment for cataract is surgery to replace the damaged lens with an artificial intraocular lens. It is one of the safest and most successful procedures in all of medicine. Surgical treatment is now a significant health cost and there are often long waiting lists; furthermore fear of surgery especially for the elderly remains an impediment to such treatment. Medical treatment of cataract is therefore a highly desired alternative.[40]

A number of calpain inhibitors have been used in studies to demonstrate the role of calpain in cataract. Leupeptin has been used in this context but it has low cell penetration due to the presence of positively charged arginine. **E-64**, another widely used calpain inhibitor, has been reported to retard the development of nuclear cataract in *in vitro*

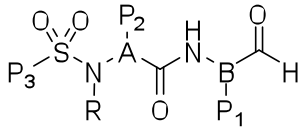
experiments. However the effect is poor in an *in vivo* animal model.[41] **SJA-6017** has been developed by Senju Pharmaceuticals Ltd and has been proved to be effective in preventing calcium-induced cortical cataract in rats.[42] Inhibitor **CAT811** has also been reported by our research group to protect sheep lens from calcium-induced cataract formation (see 1.5.2, Chapter 1).[43]

SJA-6017 has a higher potency toward calpain 1 (7.5 nM) and calpain 2 (78 nM) than other known calpain inhibitors such as **E-64**, leupeptin and **AK275** (Table 1.2).

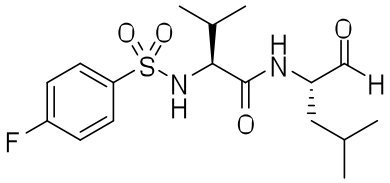
Table 1.2. Potency of **SJA-6017** compared to other known calpain inhibitors.

Compounds	IC_{50}(nM)	
	Calpain 1	Calpain 2
SJA-6017	7.5	78
E-64	570	570
Leupeptin	410	720
AK275	260	430

SJA-6017 is therefore a suitable lead compound with structure activity relationship studies[42] (Table 1.3) showing that inhibitory activity is reduced by replacing its valine with norvaline in **1.1** and norleucine in **1.2** at the P2 position. The incorporation of alanine as in **1.3**, tryptophan as in **1.4**, and phenylalanine as in **1.5** at the P1 position reduces inhibitory activity against calpain 1. This suggests that the size of hydrophobic groups at P1 and P2 is important, with Val and Leu being preferred at P1/P2 position respectively. Replacement of the aromatic ring in P3 with a methyl group (**1.6**) reduces potency. Methylation of the P2 N-H group as in **1.7** significantly reduces the inhibitory activity suggesting that the P2 N-H group is most likely required for hydrogen-bonding interactions with calpain.



SJA-6017 analogue



SJA-6017

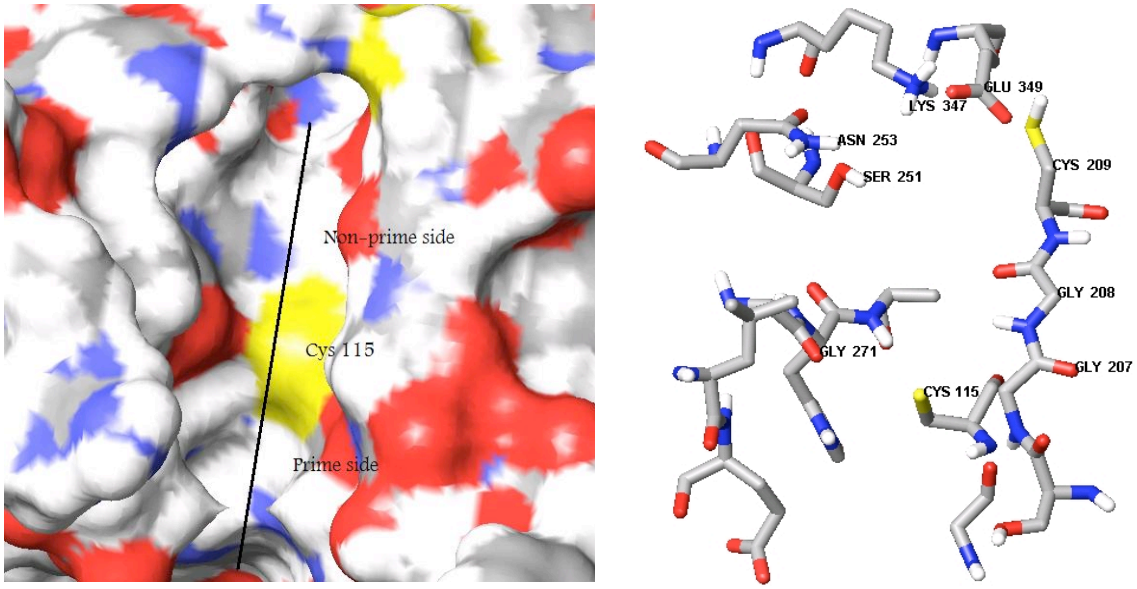
Table 1.3. Structure-activity relationship studies of lead compound **SJA-6017** and its analogues.[42]

Compound	P3	R	P2	P1	IC_{50} (nM) Calpain 1
SJA-6017	4-F-Ph	H	L-Val	L-Leu-H	7.5
1.1	4-F-Ph	H	L-Nval	L-Leu-H	130
1.2	4-F-Ph	H	L-Nle	L-Leu-H	260
1.3	4-F-Ph	H	L-Val	L-Ala-H	630
1.4	4-F-Ph	H	L-Val	L-Trp-H	23
1.5	4-F-Ph	H	L-Val	L-Phe-H	27
1.6	Me	H	L-Val	L-Leu-H	830
1.7	4-F-Ph	Me	L-Val	L-Leu-H	21000

Computer docking studies have been carried out in our research group, in an attempt to rationalize the inhibitory activity of a series of **SJA-6017** analogues and to demonstrate the validity or otherwise, of *in silico* calpain model.[44] Molecular modeling studies require knowledge of an active site and this was obtained from the recently published X-ray crystal structure of protease core of calpain 1 (1KXR)[45] and calpain 2 (1MDW)[46]. Both the 1KXR and 1MDW constructs consists of domain I and II of calpain 1 and 2 respectively.

The protease core of calpain 1 (1KXR) was crystallized in the presence of calcium and this construct has the active site cysteine mutated to serine preventing autolysis during crystallization. The protease core of calpain 2 (1MDW) in the presence of calcium has also been crystallized however this construct did not show activity in an *in vitro* assay

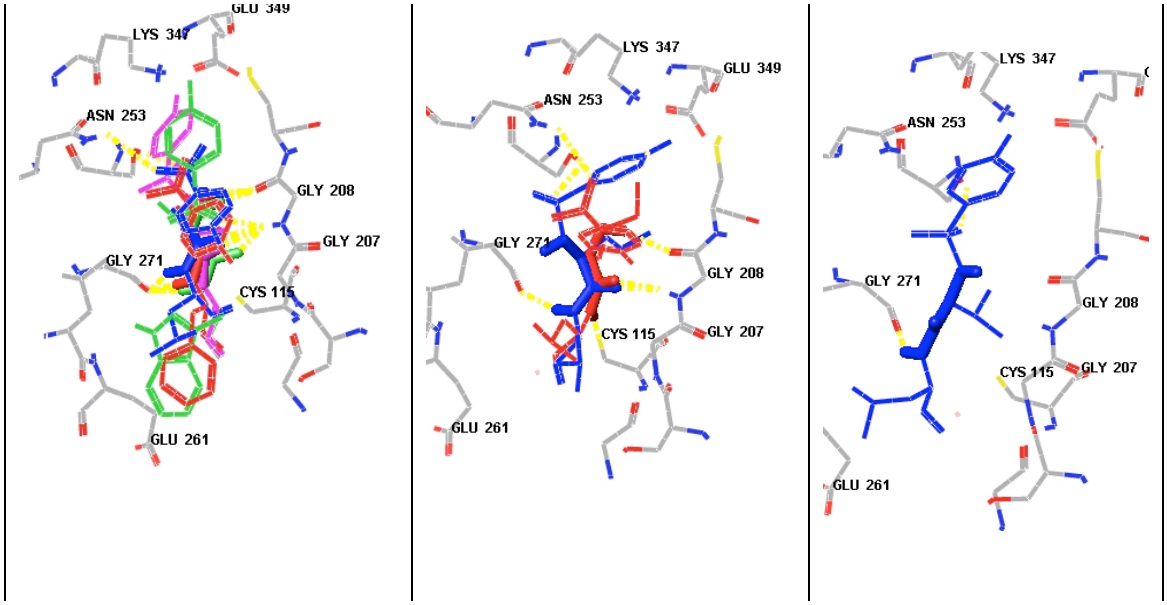
because a tryptophan is positioned over the active site.[46] Therefore the coordinates of the 1KXR construct were chosen to obtain coordinates for the active site for the molecular modelling study (Figure 1.12). To perform the docking studies, the active site serine115 was first mutated *in silico* to cysteine115, and the Cys115 deprotonated and His272 protonated to mimic the physiological conditions of action mechanism of cysteine protease.



(a) (b)

Figure 1.12. a) Surface diagram of 1KXR showing the active site cleft (black line) with the active site Cys 115 in yellow. Above the Cys 115 is the non-prime side and below is the prime side. The active site is a deep valley with high sides. b) Active site of the calpain Glide model based on the PDB structure 1KXR showing the residues surrounding the non-prime region.

The effect of varying P1, P2 and P3 residues on calpain inhibitory activity was investigated by docking studies carried out by Dr Blair G Stuart on **SJA-6017** and its analogues with the calpain construct model (Figure 1.13).



(a) (b) (c)

Figure 1.13. (a) Best docked pose of **SJA-6017** (blue), compounds **1.3** (pink), **1.4** (green) and **1.5** (red). (b) Best docked poses of compounds **1.1** (blue) and **1.2** (red). (c) Best docked poses of compound **1.7** (blue).

Dockings of **SJA-6017** (7.5 nM), **1.4** (23 nM) and **1.5** (27 nM) show three essential hydrogen bonds are formed with Gly271 and Gly208 (Figure 1.13a). The three hydrogen bonds are considered to be crucial for appropriate binding in the active site as a *β*-strand conformation. This *β*-strand conformation is universally recognized by proteolytic enzymes (see Section 1.4).[47] The best fit pose of compound **1.3** also shows these three hydrogen bonds, however compound **1.3** has one 'ugly' internal contact (see Table 1.4) reflected by an reduced inhibitory activity (630 nM) compared with **SJA-6017**, **1.4** and **1.5**. An 'ugly' internal contact reflects the molecule being forced to fit into the active site. The Glide docking program keeps the enzyme rigid and not able to move to accommodate such a ligand without unfavourable internal contacts.

Table 1.4. Docking data for best pose and inhibitory concentrations (IC_{50}) of compound **SJA-6017** and its analogues **1.1**-**1.7** with their structures shown in Table 1.3.

Compound	Glide Score	Emodel Score	Essential H bonds	Warhead Distance Å	Internal contacts			IC50 (nM) μ-calpain
					Good	Bad	Ugly	
SJA-6017	-5.8	-49.4	3	4.2	203	9	0	7.5
1.1	-4.6	-49.2	2	4.0	188	20	3	130
1.2	-4.0	-52.7	2	3.6	212	15	0	260
1.3	-4.8	-49.9	3	4.1	139	10	1	630
1.4	-4.6	-59.3	3	3.6	195	7	0	23
1.5	-5.9	-54.7	3	4.0	205	14	0	27
1.6	-5.9	-48.2	3	3.7	188	9	2	830
1.7	-2.4	-45.9	1	4.4	217	15	0	21000

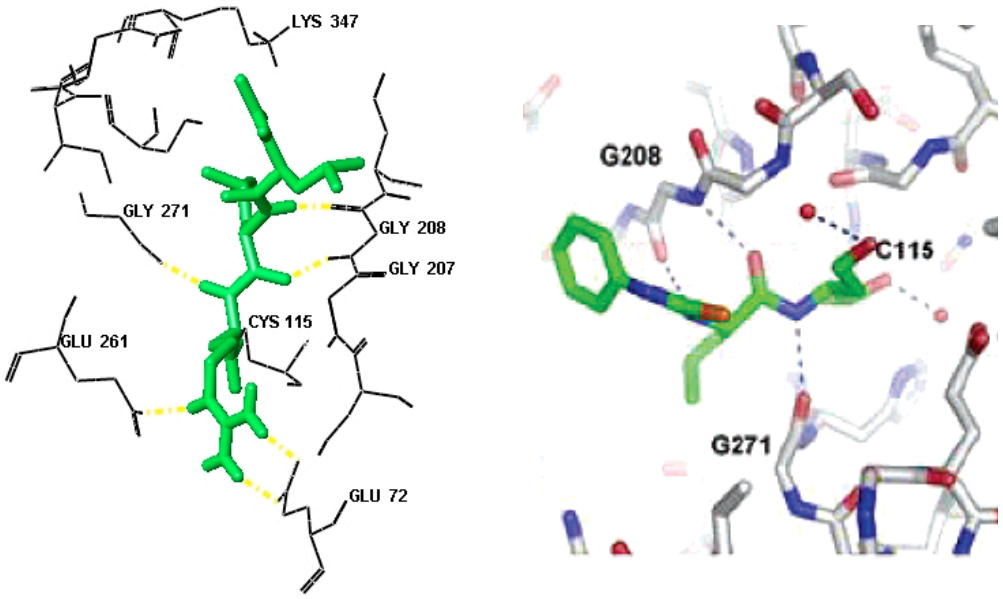
[a] War head distance (WHD) is the distance between the carbonyl carbon of the aldehyde and the active site cysteine sulfur in Å.[b] Hydrogen bonds from the carbonyl group of Gly_{208}, the NH group of Gly_{208}, and the carbonyl group of Gly_{271} of the o-CAPN1 homology model.

The best pose of **1.1** and **1.2** (Figure 1.13b) show that only two of three essential hydrogen bonds are formed, moreover compound **1.1** has a three 'ugly' internal contacts (see Table 1.4). As such this rationalises **1.1** and **1.2** exhibiting higher IC_{50} values namely 130 nM and 260 nM, respectively. Compound **1.7** (Figure 1.13c) has an only one of the three essential hydrogen bonds resulting in a poor IC_{50} of 21000 nM.

The carbonyl carbon of the warhead of all these compounds is less than 4.5 Å from the active site cysteine sulfur, a warhead distance allowing for nucleophilic attack by the sulfur of cysteine required for a reversible covalent inhibitor (Table 1.4). All these inhibitors have low negative Glide Scores and Emodel Scores suggesting tight binding (Table 1.4). Glide Score is a scoring function based on ChemScore and designed to estimate the free energy of binding for the protein–ligand complex. The function uses simple contact terms to estimate lipophilic and, where relevant, metal–ligand binding contributions, a simple explicit form for hydrogen bonds and a term which penalises flexibility. The Emodel is a model energy score that combines energy grid score, binding affinity predicted by GlideScore, and for flexible docking the internal strain energy.[48]

1.4: The universal importance of a *β*-strand conformation in protease inhibitor design

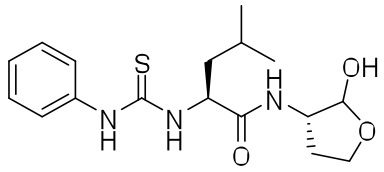
The X-ray crystal structure of leupeptin in a complex with calpain 1 construct (1TL9) (Figure 1.14a) shows that the inhibitor is covalently bound between the carbon of the aldehyde and the sulfur of Cys115. Two key hydrogen bonds formed between the NH and carbonyl groups of leucine (P2) and an additional hydrogen bond between the NH and Gly271. **SNJ-1715** complexed with calpain 1 (μI-II) also shows identical hydrogen bond interactions between the peptidyl backbone of **SNJ-1715** and residues Gly271 and Gly208 in the active site binding pocket (Figure 1.14b).



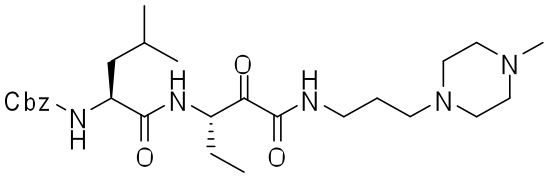
(a) **(b)**

Figure 1.14. X-ray structure of (a) leupeptin in a complex with calpain 1 construct (1TL9).[49] (b) **SNJ-1715** in the binding pocket of calpain 1 construct (2G8E).[50]

These three hydrogen bonds are also observed in the X-ray crystal structure of **E-64** in a complex with calpain 1 construct (1TL0), and **AK-295-D2** with calpain 1 construct (2R9C).[51] In all cases the result is binding of the ligand in a *β*-strand conformation.



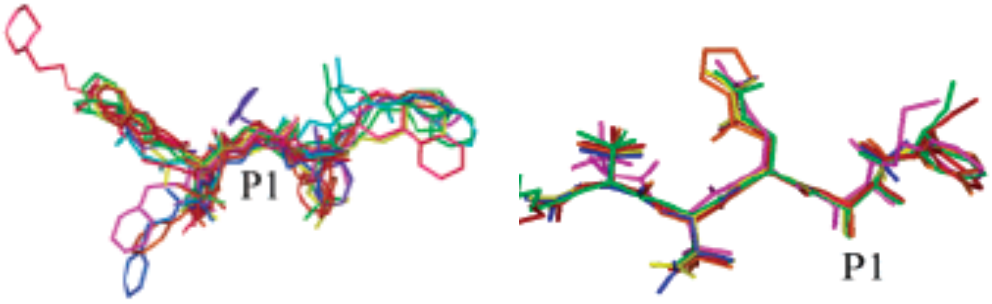
SNJ-1715



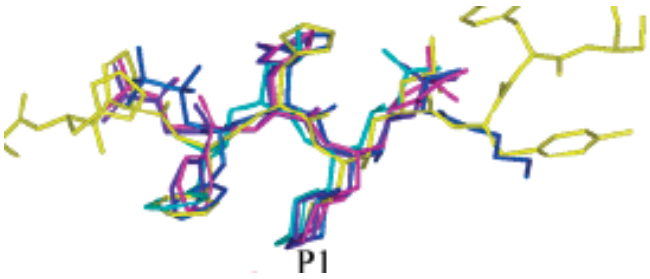
AK-295-D2

It turns out that this β-strand conformation is universally recognized by proteolytic enzymes. Over fifteen hundred X-ray crystal structures of protease-bound substrates, products and inhibitors have been reviewed in the active sites of the five protease classes; aspartic, serine, metallo, cysteine and threonine endopeptidases.[47] Peptidic and non-peptidic ligands all bind in an extended β-strand conformation, with very few exceptions.

Cysteine proteases are known to have fairly shallow, solvent exposed active sites that can accommodate a 2-4 amino acid sequence in a β-strand conformation. A total of twelve inhibitors have been shown to bind the active site of cathepsin, a cysteine protease, in a β-strand conformation (Figure 1.15a).[47] Figure 1.15b shows the overlap of the β-strand conformation of inhibitors bound to the human cytomegalovirus, a serine protease. Figure 1.15c shows the superimposition of five active-site-bound inhibitors of rennin, an aspartic protease, each in an extended β-strand conformation.



(a) **(b)**



(c)

Figure 1.15. Superimposition of (a) cysteine protease-inhibitor structure, (b) serine protease inhibitor structure, (c) aspartic protease-inhibitor structure.[47]

1.4.1: The *β*-Strand conformation

A *β*-strand is a linear or saw-toothed arrangement of amino acids in which the amide bonds are almost coplanar and the side chains alternate above and below the plane of the peptide backbone. A *β*-strand conformation for a peptide backbone can be defined by the Φ and Ψ torsion angles where -160° < Φ < -100° and 90° < Ψ < 160° (Figure 1.16). [52]

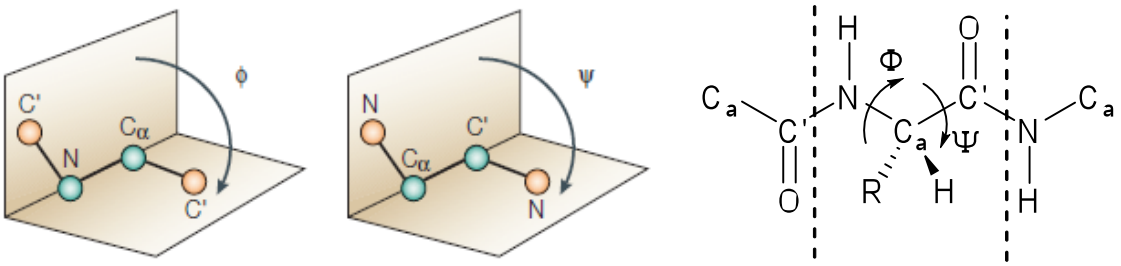


Figure 1.16. Definition of *β*-strand conformation of peptides.[53]

The positions of the amino acid side chains within an extended *β*-strand conformation are separated by a maximum distance with the i and i+4 residues which are normally 14.5 Å apart (Figure 1.17).[54] This large separation minimizes the intramolecular and steric interactions of side chains and exposes amide hydrogen-bonding atoms thereby

promoting the intermolecular interactions with the enzyme. A β-strand peptide in solution exhibits the $J_{NHC\alpha H}$ coupling constants in the range 9-10 Hz in proton NMR.

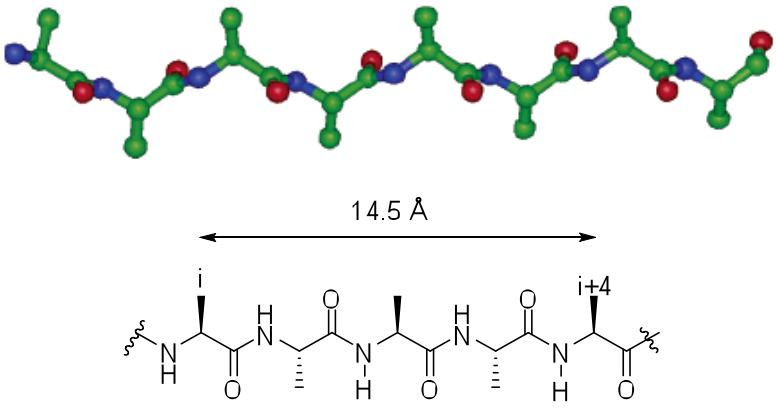


Figure 1.17. A β-strand composed of Ala residues.

Based on the observations that proteases bind their substrates and inhibitors in an extended β-strand backbone we and others have reported cyclic protease inhibitors that mimic this geometry.[43,55] Structures of this type are conformationally pre-organized into a shape that is recognized by an active site, which can increase affinity towards the enzyme by reducing the entropic penalty of inhibitor-enzyme binding.

1.4.2: β-Strand macrocyclic protease inhibitors

Macrocycle **1.8** has been reported as a potent aspartic protease BACE inhibitor (IC_{50} = 156 nM) by linking the P1 and P3 residues in acyclic BACE inhibitor 2.[56] The X-ray crystal structure (Figure 1.18) shows the macrocyclic inhibitor **1.8** in dark green overlapping with linear peptide **1.9** (orange) in an extended β-strand conformation in the active site of the aspartic protease BACE. The crucial hydrogen-bonding interactions observed in the complex of the acyclic inhibitor **1.9** with active site of aspartic protease BACE (including interactions with Gly34, Pro70, Thr72, Gln73, Gly230, and Thr232) are also observed with macrocycle **1.8**. The introduction of the intramolecular ring enhanced

the propensity of a bioactive β-strand conformation, known to favour active-site binding and thereby accounting for improved affinity for the enzyme.

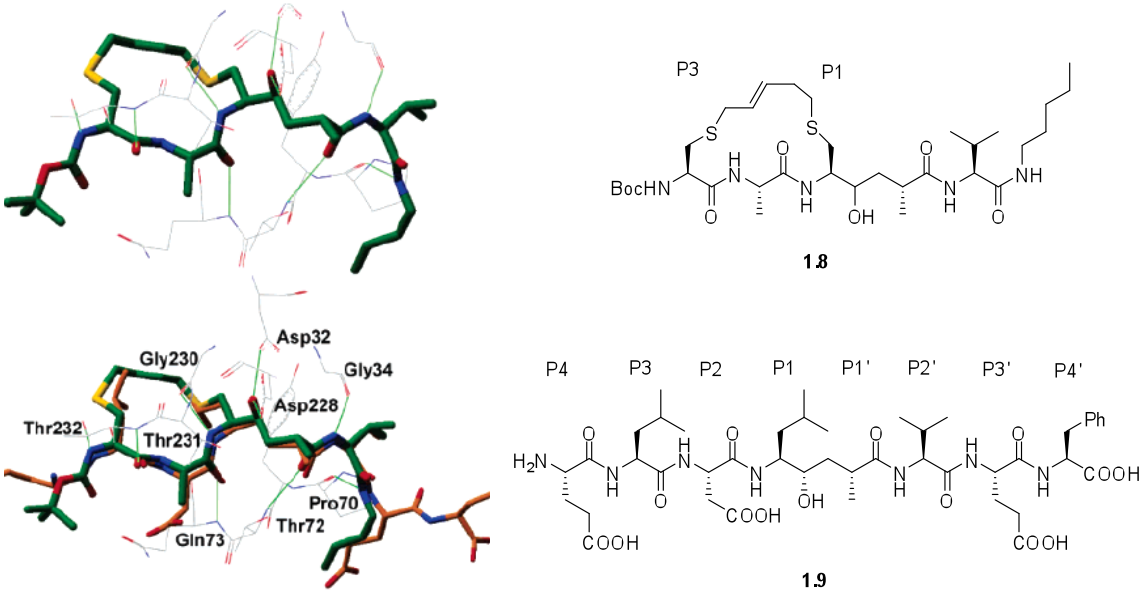
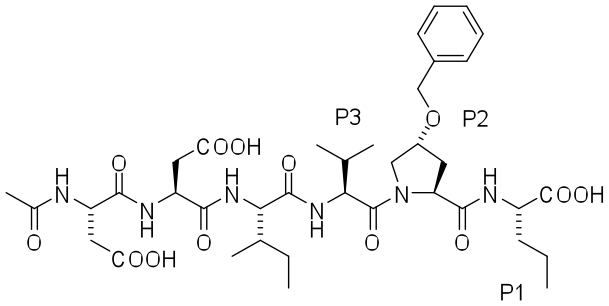
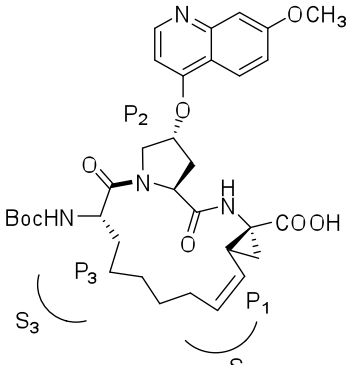


Figure 1.18. X-ray structure of macrocycle **1.8** (dark green)-BACE complex (top) and overlay of the X-ray structures of BACE complexes with macrocycle **1.8** (dark green) and acyclic **1.9** (orange) (bottom).

The linear compound **1.10** has been shown by NMR and molecular modelling to bind with HCV NS3 serine protease in an extend β-strand conformation (IC_{50} = 400 nM). Modification of **1.10** by ring closure between the of P1 and P3 side chains to a 15-membered macrocycle **1.11** with a *trans* P2 amide bond and *Z* double bond results in a potent HCV NS3 serine protease inhibitor (IC_{50} value of 24 nM).[57]

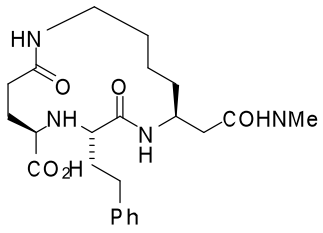


1.10

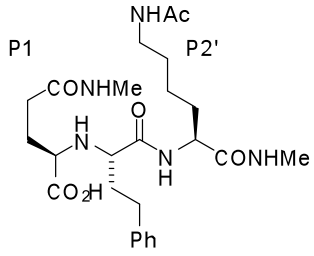


1.11 IC_{50}=24nM

The cyclic linkage of **1.12** improves the poor isoform selectivity displayed by the linear tripeptide analogue inhibitor **1.13** against matrix metalloproteinase (MMP)-8.[58] The X-ray crystal structure of inhibitor-bound MMP shows that **1.13** adopts the expected extended β-strand conformation, with the P1 and P2' side chains directed away from the active site and situated in close to each other. The X-ray crystal structure of **1.12**, a 14-membered macrocycle with linking of the P1 and P2' side chains, in a complex with MMPs shows overlap with the acyclic peptide inhibitor **1.13** in that crystal structure. The macrocycle allows the same hydrogen-bonding interactions as found for the acyclic inhibitor.



1.12



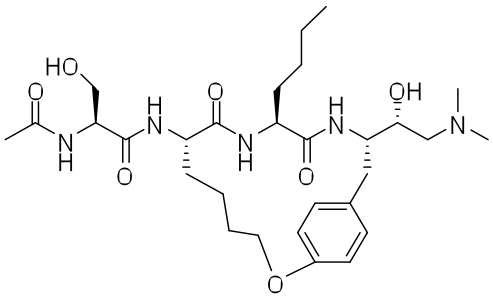
1.13

The 14-membered macrocycle **1.12** is more active to MMP-8 (17 nM) than the open-chain analogue **1.13** (293 nM) and both are selective to MMP8 over other MMP enzymes (Table 1.5).[58]

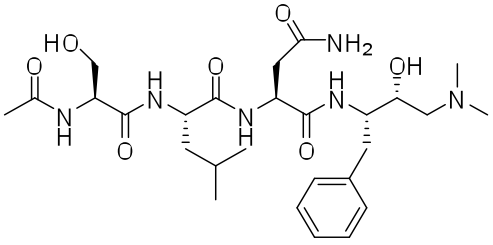
Table 1.5. Macrocycle **1.12** have a more potency and selectivity to MMP-8 than acyclic **1.13**.

K_i (nM)	14-m macrocycle **1.12**	Linear peptide **1.13**
MMP-1	2500	2860
MMP-2	8100	1533
MMP-3	13500	14088
MMP-8	17	293
MMP-9	6600	404

The 17-membered macrocycle **1.14** has been reported to mimic an extended *β*-strand conformation adopted by compound **1.15** bound to HIV-1 protease. This structure has improved potency and proteolytic stability.[59] Compound **1.15**, a tetra peptide inhibitor, is a poor drug candidate because it readily undergoes hydrolysis. Macrocycle **1.14** links the P1 and P3 side chains. The X-ray crystallographic structure of **1.14** in a complex with HIV protease shows that it forms a *β*-strand conformation similar to that observed for the acyclic peptide **1.15**. Macrocycle **1.14** is stable to proteases found in plasma or in a cell, where linear peptides are completely hydrolysed under these conditions. The amide bonds in the macrocycles are therefore in general less susceptible to cleavage than in the acyclic analogues and thus the macrocycle **1.14** exhibits favourable stability as a drug.



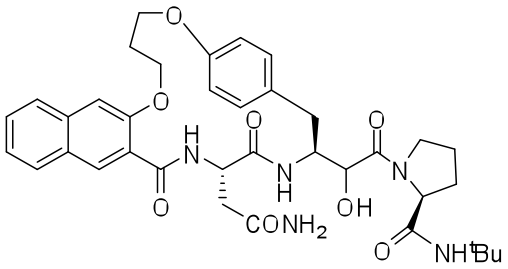
1.14



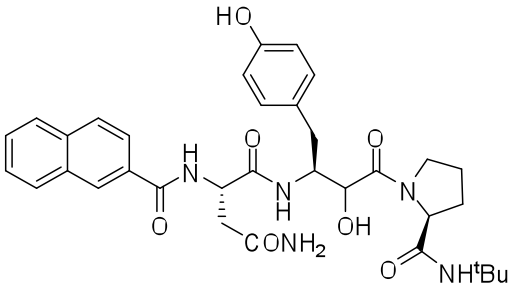
1.15

Macrocyclization also impacts on membrane permeability and cellular activity. For example, the 18-membered macrocycle **1.16**, an analogue of the acyclic inhibitor **1.17**, has improved cell permeability and inhibitory activity of cellular enzymes.[60] Compound

1.17, adopts a β-strand geometry. It exhibits sub-nanomolar inhibitory activity and is a potent antiviral compound but poor cell penetration makes this compound unsuitable as a drug.



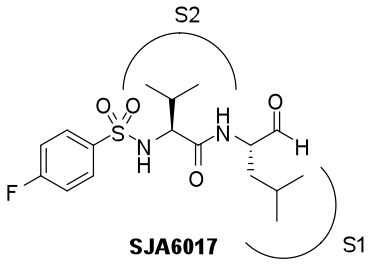
1.16

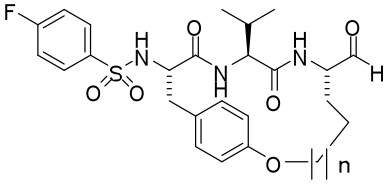


1.17

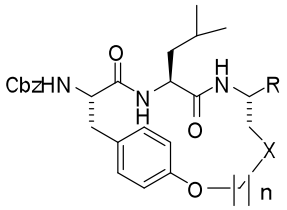
The effect of a macrocycle structure on cell permeability is apparent in a comparison of the EC_{50}/IC_{50} ratio for macrocycle **1.16** with the acyclic analogue **1.17**. This ratio for **1.16** is ca 30-fold higher than that for **1.17** showing that macrocycle **1.16** has improved cellular permeability relative to the acyclic analogue.

Macrocyclic analogues of the lead calpain inhibitor **SJA-6017**, where the core is constrained as a β-strand, also offer potential to reduce the entropic penalty of inhibitor-enzyme binding and to improve pharmacokinetic properties such as target affinity, membrane permeability, enzyme selectivity, stability to proteolysis. In particular, the 16-19 membered macrocycles **1.18-1.23** have been reported by our research group to be constrained with a β-strand core.[43] Compounds **1.18** and **1.19** incorporate the Val-Leu dipeptide backbone of **SJA-6017** but in a macrocycle by linking the P1 leucine and P3 tyrosine side chains. Compounds **1.20-1.23**, which have the 4-fluorobenzyl sulphonamide of **1.18** and **1.19** replaced with a Cbz group and the P2 valine of **SJA-6017** replaced by Leucine, have been reported. These compounds have been studied because it was thought the macrocyclic structure may increase potency and bioavailability. Alcohols **1.20b-1.23b** are precursors to aldehydes **1.20a-1.23a**.





1.18: n=1
1.19: n=2



1.20: n=1,16m, X=CH_2 **a:** R=CHO
1.21: n=2,17m, X=CH_2 **b**: R=CH_2OH
1.22: n=3,18m, X=CH_2
1.23: n=4,19m, X=S

The assay results of **1.18-1.23** with ovine calpain 2 shows that the 17-membered macrocyclic aldehyde **1.21a** (**CAT811**) is most potent against *o*-calpain 2, with an IC_{50} of 30 nM.[43] This compound significantly retards calcium-induced opacification in an *in vitro* ovine lens culture assay and shows promise in slowing the progression of cortical cataract in animal trials with a hereditary ovine cataract model.[61] Aldehydes **1.19** (280 nM) and **1.22a** (180 nM) also proved to be potent with nanomolar inhibitory activity. Interestingly the 17-membered alcohol **1.21b**, a precursor to **1.21a**, shows potency with the IC_{50} value of ca. 700 nM.[43]

1.5: Metathesis and its use in the synthesis of macrocyclic protease inhibitors

The macrocycles of the protease inhibitors **1.18-1.23** above were introduced by ring closing metathesis of a suitable diene precursor, a sequence discussed in detail in this thesis. Such alkene metathesis[62] was discovered in mid-1950 by researchers studying Ziegler polymerization.[63] Its subsequent wide applicability to organic synthesis has resulted from the discovery by Schrock[64] and Grubbs[65] of well defined and function-group-tolerant metathesis catalysts.

Alkene metathesis can be classified into four categories: cross metathesis (CM), ring closing metathesis (RCM), ring opening metathesis (ROM) and ring opening metathesis polymerization (ROMP) (Figure 1.19). CM forms an acyclic carbon-carbon bond with an intermolecular mutual exchange of carbene fragments between the two alkenes. RCM involves intramolecular reaction of two alkenes with catalyst to generate medium-to-large

ring architecture. Ring opening metathesis is a process through which a cyclic alkene reacts with an acyclic alkene to give a new acyclic diene. The driving force for ring opening metathesis is the release of ring strain. Finally, ROMP also requires a constrained cyclic structure and ring open to give a diene with terminal double bonds complexed with a metal, the metal-carbon carbene reacts with the double bond on the next monomer, thus propagating the reaction to produce linear polymers.

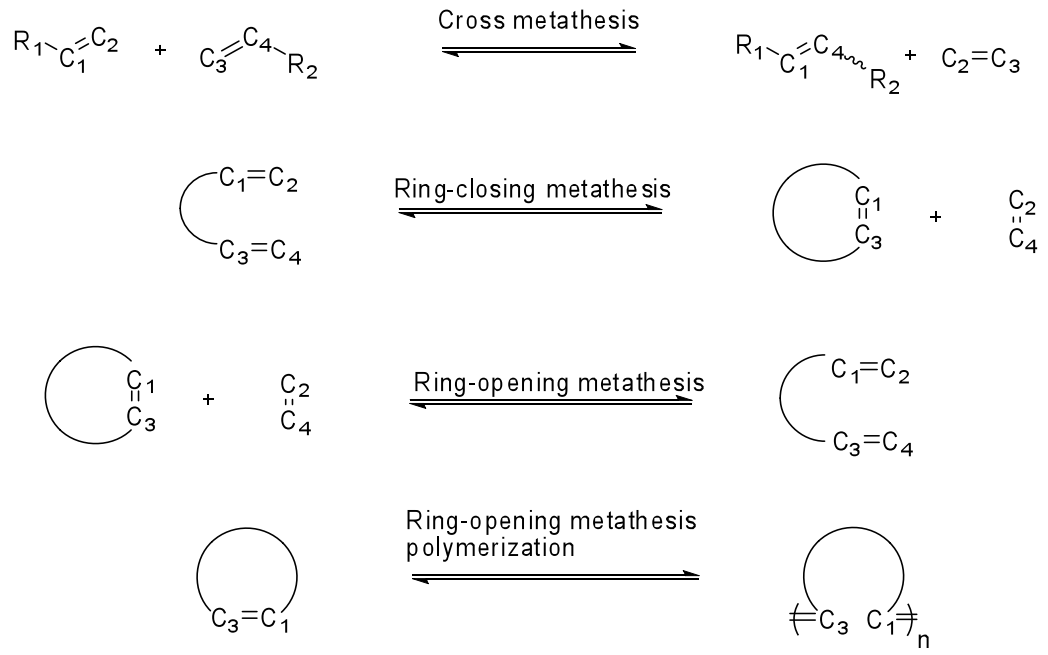


Figure 1.19. Main types of alkene metathesis: CM, RCM, ROM and ROMP.

As a carbon-carbon double bond forming reaction alkene metathesis has distinct advantages:

(i) The alkene substrates can often be easily prepared;

(ii) The reaction conditions tolerate a wide range of functional groups;

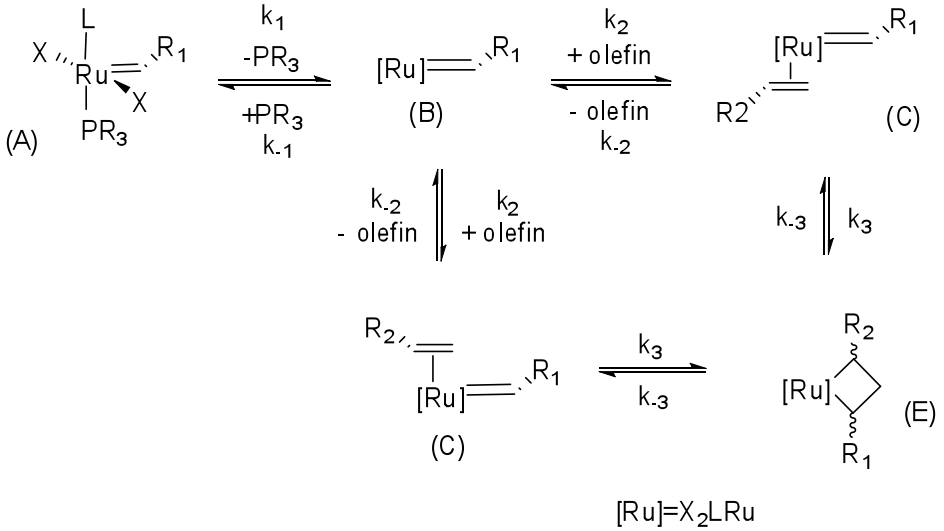
(iii) Alkene products allow for further functionalisation;

(iv) Alkene metathesis often provide product in high yield;

(v) Ethylene is often a byproduct of metathesis and is easily removed.

1.5.1: Development of alkene metathesis catalyst

Advances in the synthesis of transition metal complexes led to the synthesis of a numerous metal organic carbene complexes. Selected complexes of titanium, tungsten, molybdenum and ruthenium have been shown to effect metathesis reactions of alkenes. The metal-carbon double bond of the complex, often referred to as a carbene complex, e.g. Mo=C, Ru=C, reacts with alkenes as shown in **Scheme 1.1**.



Scheme 1.1. Mechanism of ruthenium-catalyzed alkene metathesis.

In catalytic alkene metathesis, the presence of functional groups (such as oxygen, heteroatoms) or solvents (including oxygen and water) can interfere with catalytic activity by binding to the active metal center or reacting directly with the metal center. Therefore the development of metathesis catalysts required use of a metal that can react preferably with alkene and tolerate other reactive functional groups. A selection of metathesis catalysts is shown in Figure 1.20 along with their tolerance of functionality in the reacting systems and a comparison of their reactivity.

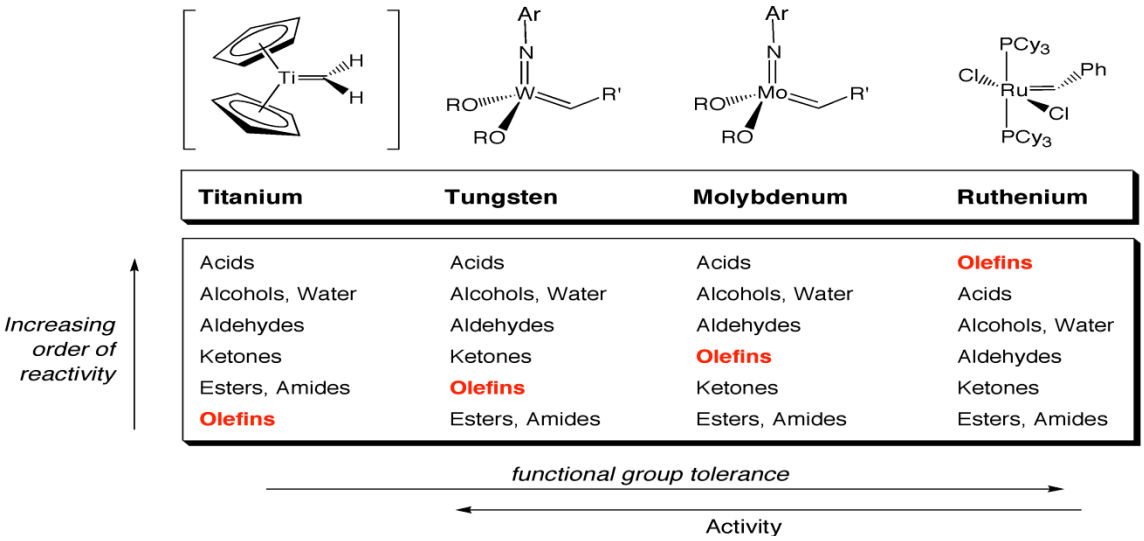


Figure 1.20. Metal center functional groups.[66]

Although titanium and tungsten based carbene catalysts promote alkene metathesis, their use has been limited because of their low stability and reactivity. Molybdenum-based carbene complexes are more reactive towards alkenes but also react with aldehydes and polar or protic groups. Ruthenium is the metal of choice because ruthenium-based carbene complexes react preferably with alkenes over other functional group and tolerate many functional groups including carboxylic acids, alcohols and aldehydes and ketones, but are rendered inactive in the presence of amides. Ruthenium catalysts with the general formula $(PR_3)_2(X)_2Ru{=}CHR_1$ are commonly used because of their air stability and ease of handling.

A mechanism of ruthenium-catalyzed alkene metathesis (Scheme 1.1) requires phosphine (PR_3) dissociation to form active 14-electron species **B**. which can either reform **A** with a rate constant k_{-1} or react with alkene with a rate constant k_2 to give complex **C** and metallacyclobutane **E**. The geometry of intermediate **C** and **E** can be *cis* or *trans*.

The effect of ligand L, substituents X, R and R1 on the activity of ruthenium-based catalysts **1.24** has been investigated (Figure 1.21).[67] The air stable complex **1.25** has been found to be an effective catalyst for polymerization of norbornenes in protic media. Although **1.25** is not particularly active, its well-defined structure provides a scaffold by

which to make structural changes for catalyst optimization. The activity of the catalyst **1.25** was shown to be increased by replacing PPh_3 with PCy_3 (**1.26**).

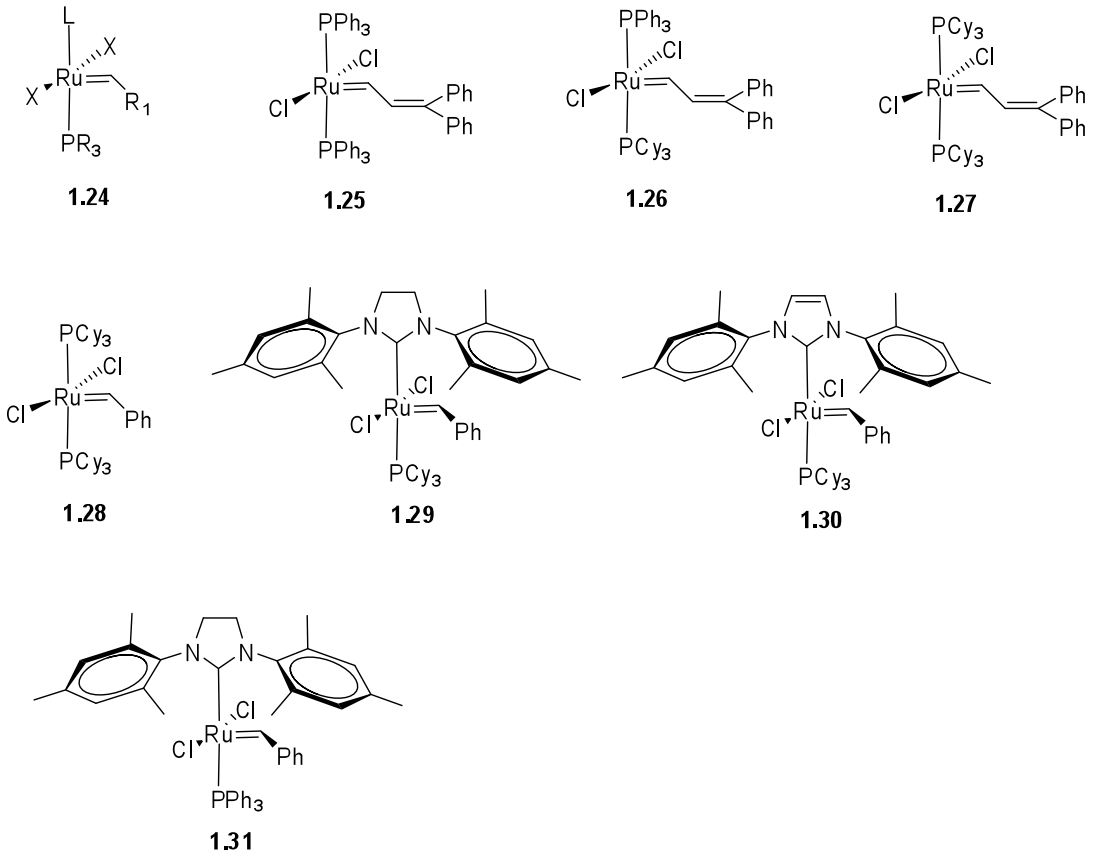


Figure 1.21. Ruthenium-based alkene metathesis catalysts.

The ligand R_1 also influences the phosphine dissociation rate k_1. For example, changing the carbene ligand from $CHCHC(Ph)_2$ in **1.27** to CHPh in **1.28** results in an increase in k_1 and this can be rationalized on the basis of the steric and electronic nature of the R_1 substitute. The CHPh group is sterically bulky and less electron withdrawing than $CHCHC(Ph)_2$ thereby promoting phosphine dissociation and leading to a high dissociation rate.

The rate of metathesis increases on changing the ligand L from a phosphine ligand in **1.28** to N-heterocyclic carbene (NHC) in **1.29**. This increase is attributed to electron

donating ability and the steric bulk of the NHC ligand. Similarly $IMesH_2$ catalyst **1.29** is more active than IMes complex **1.30** because the $IMesH_2$ ligand is a better electron donor than IMes.

Phosphine ligands also affect catalyst activity. For example, replacing the PCy_3 of catalyst **1.29** with PPh_3 results in a more active catalyst **1.31** and this is considered to be related with the lower basicity of the PPh_3 ligand relative to PCy_3. The less electron donating phosphine ligand is expected to be more liable, facilitating the equilibrium to the active 14 electron form of the catalyst.

1.5.2: Ring closing metathesis

A general mechanism for ring closing metathesis (RCM) is shown in Figure 1.22. Reaction between an active carbene $[M]=CH_2$ and one of the two alkenes of **i** results in a metallacyclobutane **ii**, which can give a metal-carbene intermediate **iii**. Addition of the carbene to the other alkene gives a metallacyclobutane **iv** and subsequent fragemenataion gives **v** and regenerates catalyst $[M]=CH_2$ which can propagate additional reaction cycles.

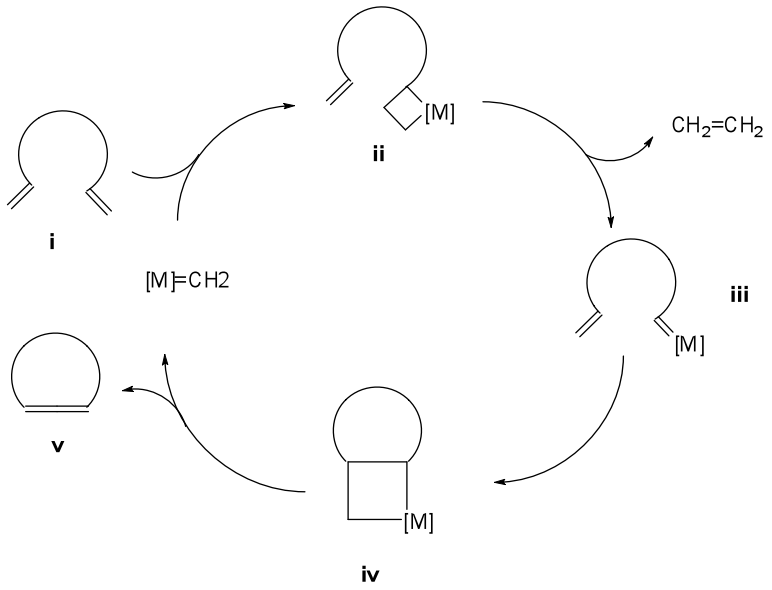


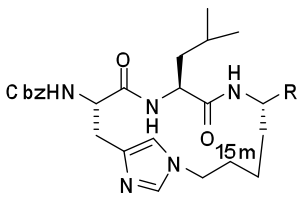
Figure 1.22. Mechanism of RCM.

1.6: Overview of the work reported in this thesis

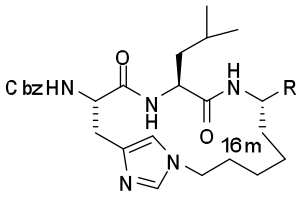
The yield of RCM used to prepare the macrocyclic compounds **1.18-1.23** is generally at best, moderate.[43] In chapter 2, the factors that influence the efficiency of such RCM reactions are investigated including the metathesis catalyst, solvent, reaction temperature, and the addition of Lewis acids. A series of diene precursors to **1.20-1.22**, has been synthesized and RCM under various conditions investigated. The results of this study have recently been published.[68] The best reaction conditions developed were then used to synthesize a most potent calpain inhibitor **1.21a** (**CAT811**).

An efficient large scale synthesis of macrocyclic aldehyde **CAT811** that does not involve RCM was also investigated (see Chapter 3).[69] This synthetic route employs an intramolecular nucleophilic substitution for ring closure which is particularly suited to a multi-gram synthesis. This intramolecular nucleophilic substitution methodology was also used in an attempted synthesis of a 19-membered macrocycle **3.21**.

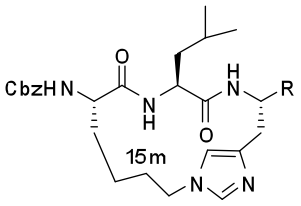
In Chapter 4, the synthesis, biological assay and molecular modelling of imidazole-containing macrocycles **4.1-4.3** are discussed. The 15- and 16-membered macrocycles **4.1** and **4.2**, with a histidine at P3, have been shown to adopt *β*-strand conformation when docked with our modeling methodology. Another 15-membered macrocycle **4.3**, with histidine in the P1 position, was also prepared in an attempt to develop some structure-activity relationships and to investigate the validity of the modelling.



4.1



4.2



4.3

a: R=CO_2Me
b: R=CH_2OH
c: R=CHO

Several intramolecular macrocyclisation strategies were investigated for the synthesis of the histidine containing macrocycles **4.1-4.3**, with intramolecular lactamization proving to be the most efficient (see Chapter 4).

RCM is generally carried out in organic solvents. In chapter 5 we report RCM reactions of a ruthenium catalyst containing a polyethylene glycol ligand in aqueous media (see Chapter 5).

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Chapter 2: The synthesis of macrocyclic β-strand templates by ring closing metathesis

2.1: Introduction

Ring closing metathesis (RCM) has been widely used in the field of peptide synthesis and peptidomimetics to produce macrocyclic molecules. The incorporation of a macrocyclic ring in an acyclic peptide analogue can lead to desirable conformational and pharmacological properties as discussed in the previous chapter.[1] For example, macrocyclization using RCM has been used as a strategy to produce mimics of protein structures including an extended β-strand, a conformation universally adopted by substrates or inhibitors for binding to proteases (see Chapter 1).[2]

The resulting macrocyclic peptides (Figure 2.1) are characterized by a number of parameters including:

(i) the number (m) of atoms in the macrocycles;

(ii) the presence or absence of carbon carbon double bonds;

(iii) the configuration of the double bond in the bridge;

(iv) the position and nature of constituent heteroatoms, multiple bonds, stereogenic centers and side chains within and on the bridge;

(v) the sequence of amino acids in the parent peptide chain;

(vi) the atoms e.g. C^{α}, C^{β}, N or carbonyl carbon of the parent peptide backbone at which the alkene functionality was introduced.

(vii) the positions of C^{α}, C^{β}, N or carbonyl carbon in the peptide backbone (e.g. i and $i+k$).

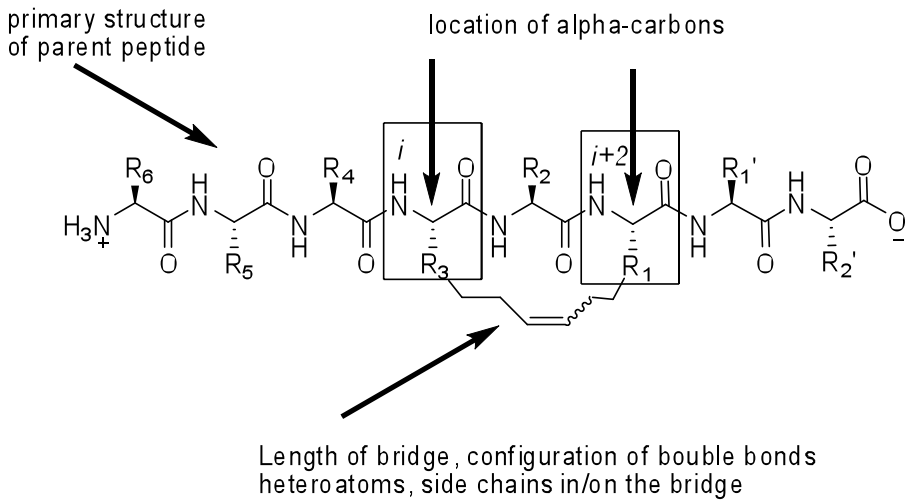
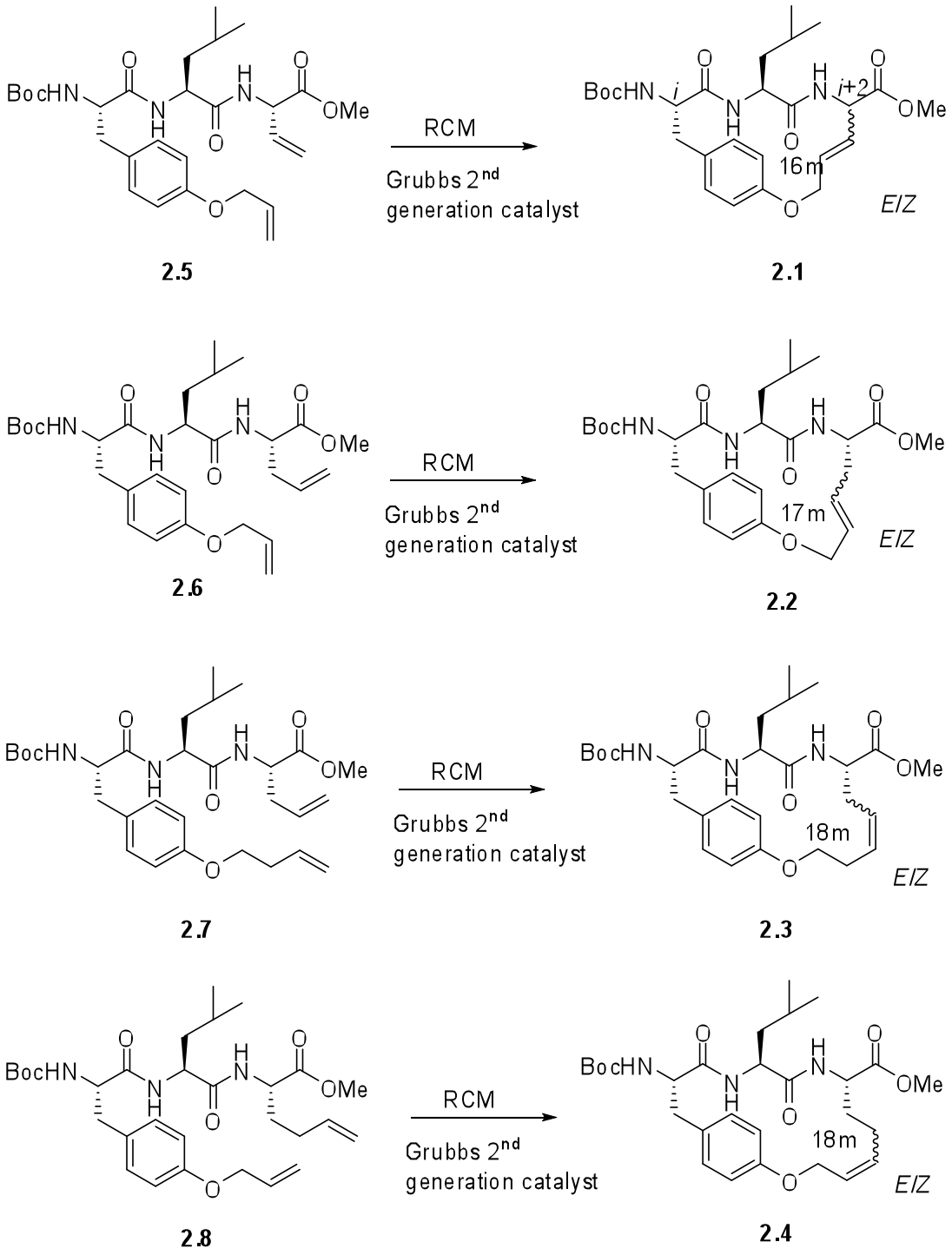


Figure 2.1. Classification of macrocyclic peptides

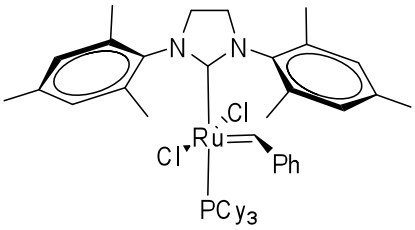
This chapter reports the synthesis of the macrocyclic β-strand templates **2.1-2.4** using RCM of dienes **2.5-2.8** respectively (Scheme 2.1).

The vinyl and allyl groups of the key dienes **2.5**, **2.6** and **2.7** can be introduced using commercially available (*S*)-vinyl-Gly-OMe and (*S*)-allyl-Gly-OMe. The allyl substituted tyrosine derivatives of **2.5**, **2.6**, and **2.8** were introduced using commercially available *N*-Boc-Tyr(*O*-allyl)-OMe. The *O*-homoallyl *N*-Boc-tyrosine used in the preparation of **2.7** was prepared by reaction of tyrosine with 4-bromobut-1-ene (see Scheme 2.4). The homoallyl-substituted glycine required for the preparation of **2.8** was prepared by the method of Fairlie[3] (see Scheme 2.5). Incorporation of these resulting building blocks into the peptide backbone gave the acyclic RCM precursors **2.5-2.8**.



Scheme 2.1. RCM of dienes **2.5-2.8**.

These dienes were subjected to RCM using Grubbs 2nd generation catalyst to give the desired alkene macrocycles **2.1-2.4** as *E*/*Z* isomeric mixtures as determined by ^{1}H NMR spectroscopy. RCM is known to be influenced by solvent, temperature and by the presence of Lewis acid and as such various reaction conditions were investigated in an attempt to improve yields.



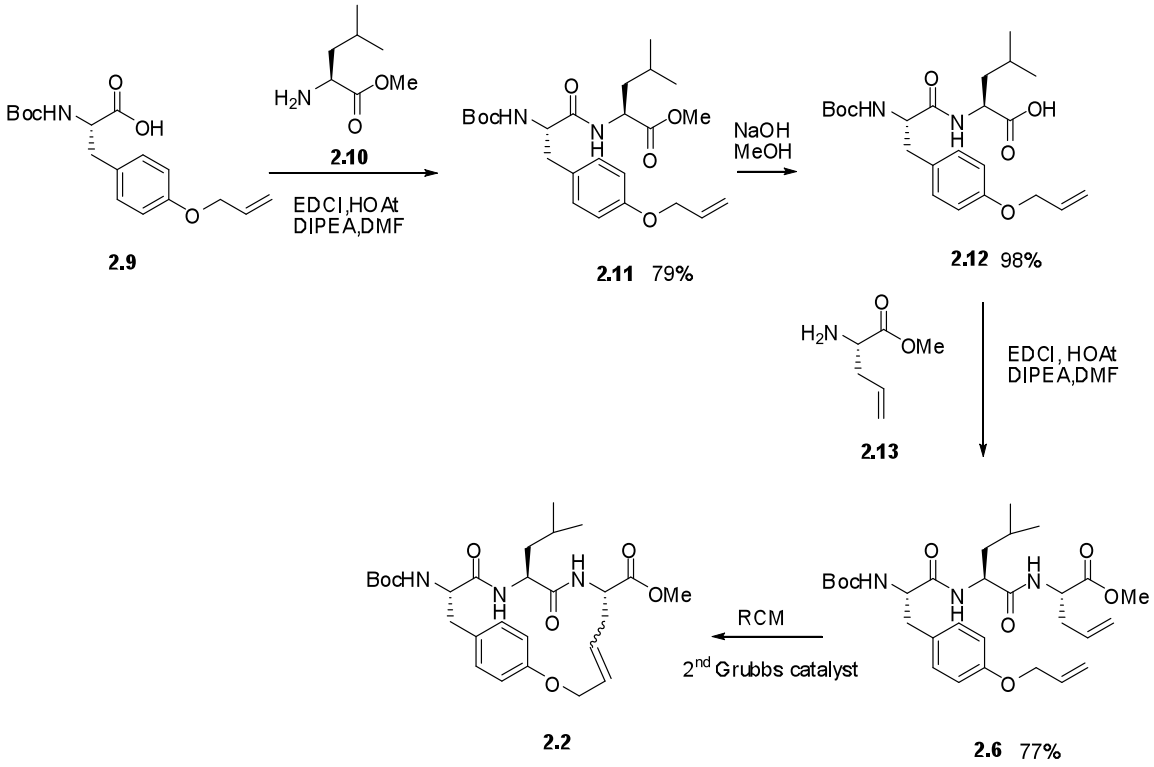
Grubbs second generation catalyst

2.2: Synthesis of 17-membered macrocycle 2.2

2.2.1: Preparation of diene 2.6

The detailed synthesis of 17-membered macrocycle **2.2** is shown in Scheme **2.2**. EDCI (1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride) catalysed coupling of the commercially available allylic ether **2.9** with Leu-OMe **2.10**, in the presence of 1-hydroxy-7-aza-benzotriazole (HOAt) as a peptide coupling reagent, gave dipeptide **2.11** (79%). The molecular formula of **2.11** was confirmed by mass spectrometry with a parent ion at m/z 449.2655 and by ^{1}H NMR spectroscopy with two resonances at 4.54 and 4.30 ppm corresponding to α-protons of leucine and tyrosine, respectively. Hydrolysis of the methyl ester of **2.11**, under basic conditions, gave carboxylic acid **2.12** in almost quantitative yield. A EDCI mediated coupling of **2.12** with commercially available (*S*)-allyl-Gly-OMe **2.13** gave **2.6** in 77% yield, with its structure confirmed by the presence of three α-proton resonances for the leucine, allylglycine and tyrosine residues centered at

4.53, 4.43 and 4.33 ppm, respectively. RCM of diene **2.6** was then carried out and factors that influence the efficiency of the reaction investigated.



Scheme 2.2. Synthesis of 17-membered macrocycle **2.2** by RCM.

2.2.2: Investigation of conditions for RCM of diene 2.6 to macrocycle 2.2

The most commonly used solvents in Ru-catalyzed RCM include dichloromethane, toluene, benzene, and 1,1,2-tichloroethane (TCE). TCE was selected as the solvent of choice for all the RCM reactions described herein because of its frequent use in metathesis reactions in combination with Ru catalyst including reactions under microwave conditions [4]

It is generally acknowledged that heating is required to initiate the dissociation of the catalyst to generate the reactive metal carbene. For a ruthenium based catalyst, decomposition is generally observed at temperature above 50 ^{o}C within a few hours.[5] RCM of diene **2.6** was first carried out in TCE (bp 60.3 ^{o}C) with three successive additions of 10 mol % Grubbs 2nd generation catalyst under reflux conditions (A, in Table 2.1).

Table 2.1. RCM of diene **2.6** under varying conditions.

Diene	Condition [a]	Product	Ratio[b]	Yield [c]
2.6	A	**2.2**	10(*E*):1(*Z*)	22%
	B	**2.2**	9(*E*):1(*Z*)	37%
	C[d]	**2.2**	9(*E*):1(*Z*)	34%
	C[e]	**2.2**	9(*E*):1(*Z*)	82%
	D	**2.2**	9(*E*):1(*Z*)	90%

[a] All reactions were performed in TCE with 3 separate additions of 10 mol % Grubbs 2nd generation catalyst. Condition A: Thermal reflux 18 h. Condition B: Microwave reflux 1 h. Condition C: Thermal reflux with Lewis acid 18 h. Conditions D: Microwave reflux with Lewis acid 1 h. [b] RCM metathesis result in mixtures of geometric isomers as confirmed by ^{1}H NMR spectroscopy. [c] Isolated yields following column chromatography. [d] Titanium-based Lewis acid. [e] chlorodicyclohexylborane

Under these conditions, the product **2.2** was isolated in 22% yield as a mixture of *E* and *Z* alkenes (10:1 by ^{1}H NMR) after chromatography on silica gel. The molecular formula was confirmed by the parent ion in the mass spectrum at m/z 518.2769. The configuration of major product alkene in the mixture was established by ^{1}H NMR analysis. This isomer exhibited a coupling constant of J = 15.5 Hz for the Ha and Hb protons, which is consistent with an (*E*) configuration[6] (Figure 2.2).

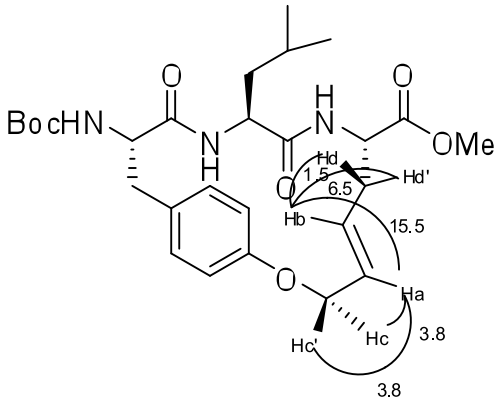


Figure 2.2. Coupling constants (Hz) of the alkenic protons in macrocycle **2.2**

The ^{1}H NMR spectra of **2.2** (Figure 2.3) shows the alkene proton Ha centered at δ 5.54 ppm as an app dt (J = 15.5, 3.8, 3.8 Hz) and Hb centered at δ 5.44 ppm as ddd (J = 15.5, 6.5, 1.5 Hz). The two methyl ester resonances were centered at 3.757 and 3.779 ppm in a ratio of 10:1. The major isomer is the *E* alkene, and the minor isomer is considered to be the *Z* isomer but it was not possible to identify the alkene protons in the NMR spectrum.

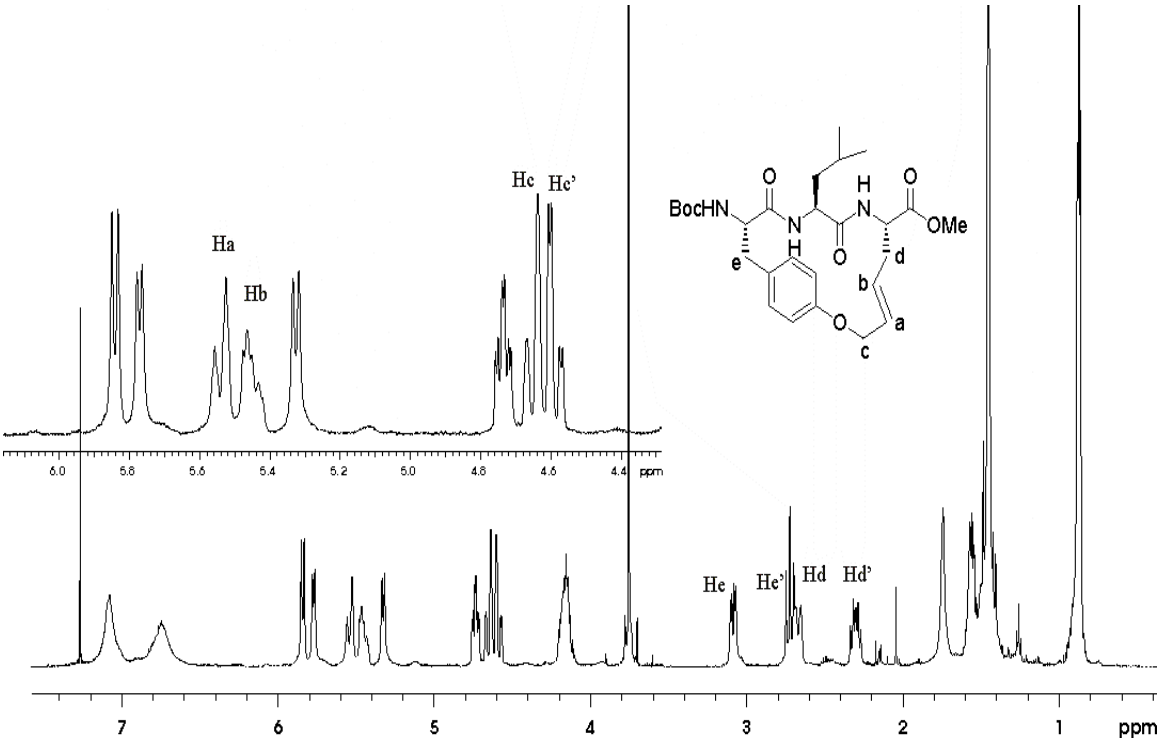


Figure 2.3. ^{1}H NMR spectrum of alkene macrocycle **2.2**.

Microwave irradiation has been reported to increase the yield in RCM reactions and to reduce reaction times.[7] Thus RCM of **2.6** was carried out with the same catalyst loading as before (3 x 10 mol %) but under microwave conditions (condition B, Table 2.1). This resulted in an improved yield of **2.2** (37%) with an *E*/*Z* alkene isomer mixture of 9:1. Thus both thermal reflux and microwave-assisted conditions gave macrocycle **2.2** in moderate yields, 22% and 37% respectively. Attempts were made to improve the yield. It has been reported that the presence of polar functional groups (e.g. ester, ketone, ether, urethane) within dienes can be detrimental to RCM due to associated coordination of the ruthenium carbene.[8] This led us to propose that ruthenium carbene in diene **2.6** might coordinate with carbonyl oxygen to form a stable 6-membered chelate structure (Figure 2.4), thus sequestering the catalyst in an unproductive complex.

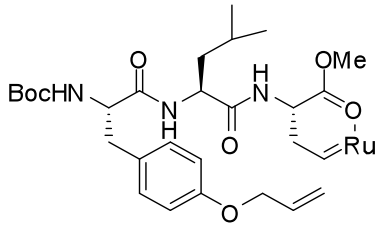


Figure 2.4. Structure of a six-membered chelate.

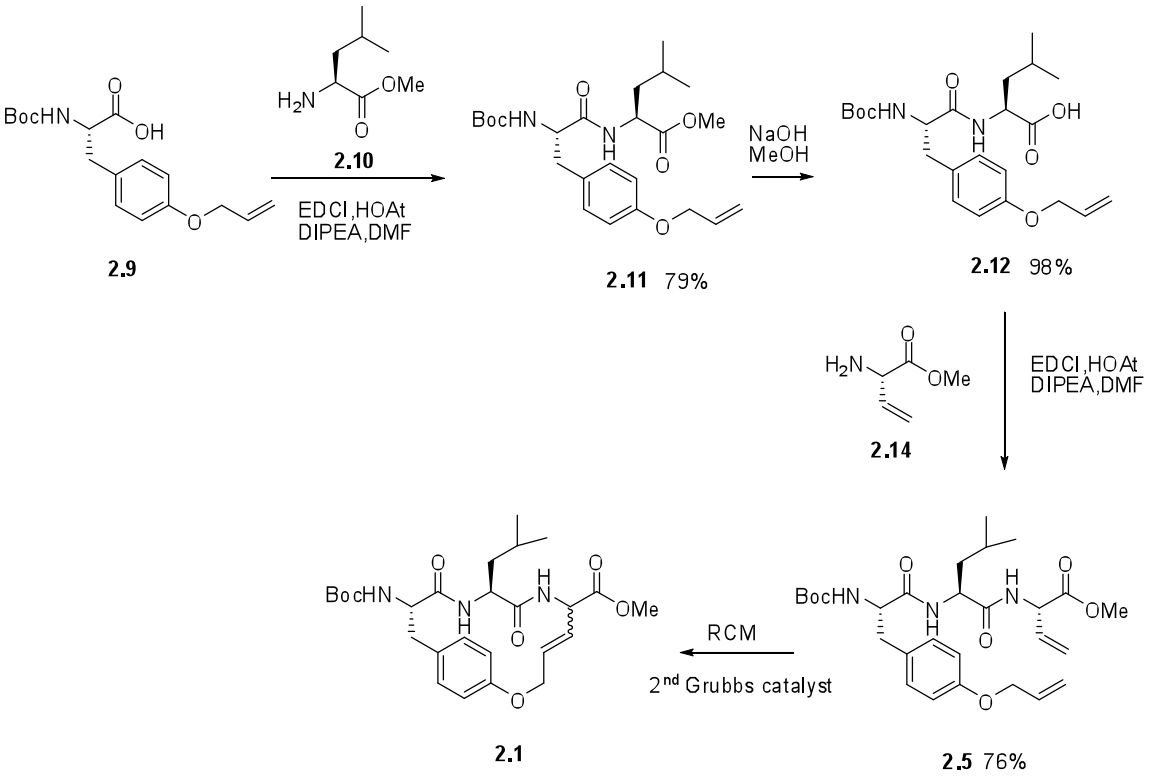
Fürstner has reported[9] that the addition of the Lewis acid $Ti(OiPr)_4$ can increase the yield of alkene metathesis in the cases where the dienes carry an ester functionality. The Lewis acid presumably functions by suppressing the formation of an unproductive chelate. Alkoxides of Ti^{4+} species can undergo kinetically labile coordination with an ester moiety, and the introduction of the Lewis acid $Ti(OiPr)_4$ to the reaction system may prevent coordination of carbonyl oxygen with ruthenium carbene intermediate and thereby suppress the formation of such a carbene chelate. RCM of diene **2.6**, in the

present of 10 mol% of $Ti(OiPr)_4$ under reflux (conditions C[d], Table 2.1), resulted in a slight increase in the yield of **2.2** to 34%.

Vedrenne[10] has studied the effect of a number of Lewis acids on olefin metathesis. This study found that while titanium Lewis acid was unsatisfactory, the addition of chlorodicyclohexylborane (a boron-based Lewis acid) significantly improved the yield of a metathesis reaction. RCM of **2.6** was therefore performed in the presence of a borane-based Lewis acid (under thermal reflux) with three sequential additions of Grubbs 2nd generation catalyst (conditions C[e], Table 2.1). The addition of chlorodicyclohexylborane resulted in a significant increase in yield of **2.2** (82%), with the similar *E/Z* alkene isomer ratio of 9:1. The use of the borane-based Lewis acid under microwave-assisted conditions (see conditions D) gave further improvement in yield (90%).

2.3: Synthesis of 16-membered macrocycle 2.1 *via* RCM under conditions A-D

The synthesis of the alkene macrocycle **2.1** was attempted by RCM of diene **2.5** under conditions A, B, C and D. The diene **2.5** was prepared from commercially available *N*-Boc-Tyr-OMe **2.9** as shown in Scheme 2.3. Reaction with Leu-OMe **2.10** in the presence of EDCI and HOAt gave the dipeptide **2.11**. The methyl ester was then hydrolysed under basic conditions to give the acid **2.12**. This was coupled with commercially available (*S*)-vinyl-Gly-OMe **2.14**, in the presence of EDCI and HOAt, to give **2.5** (76%). The molecular formula of **2.5** was confirmed by a parent ion in the mass spectrum at m/z 532.3027 and its structure confirmed by ^{1}H NMR spectroscopy.



Scheme 2.3. Synthesis of 16-membered alkene macrocycle **2.1** by RCM.

RCM of **2.5** under conditions A (Table 2.2) gave the macrocycle **2.1** as an *E*/*Z* mixture in moderate yield of 49% after purification by column chromatography. A mass spectrum of the product confirmed the expected molecular formula with a parent ion at m/z 504.2727. The configuration of the major alkene isomer was established by the ^{1}H NMR spectroscopy. This revealed a vinylic coupling constant of J = 15.5 Hz, which is again consistent with the (*E*) configuration (Figure 2.5).

Table 2.2. RCM of diene **2.5** under different conditions

Diene	Condition [a]	Product	Ratio[b]	Yield [c]
2.5	A	**2.1**	19(*E*):1(*Z*)	49%
	B	**2.1**	20(*E*):1(*Z*)	58%
	C	**2.1**	19(*E*):1(*Z*)	50%
	D	**2.1**	20(*E*):1(*Z*)	47%

[a] All reactions performed in 1,1,2 TCE with 3 separate additions of 10 mol % Grubbs 2nd generation catalyst. Condition A: Thermal reflux 18 h. Condition B: Microwave reflux 1 h. Condition C: Thermal reflux with borane-based Lewis acid 18 h. Conditions D: Microwave reflux with Lewis acid 1 h. [b] RCM results in mixtures of geometric isomers. [c] Isolated yields following column chromatography.

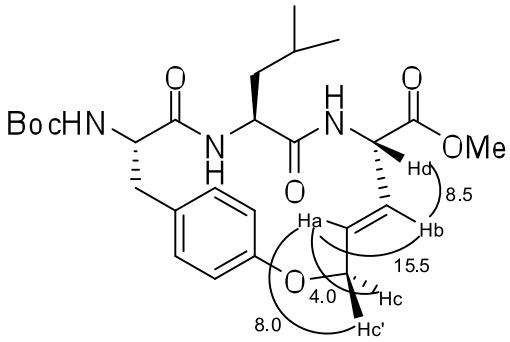


Figure 2.5. Coupling constants (Hz) of alkenic protons in macrocycle ***E*-2.1**

The mixture revealed two methyl ester resonances in a ratio of 19:1. These are centered at 3.786 ppm and 3.747 ppm and correspond to the major and minor alkene, respectively. The Ha proton on the double bond centered at 5.72 ppm appears to be a ddd (J = 15.5, 4.0, 8.0 Hz) and the alkene Hb centered at 5.42 ppm was shown as dd (J = 15.5, 8.6 Hz). The coupling constants observed for alkenic protons Ha and Hb (J = 15.5 Hz) reveal the major isomer of **2.1** is the (*E*) isomer. The corresponding signals for the minor isomer were not resolved.

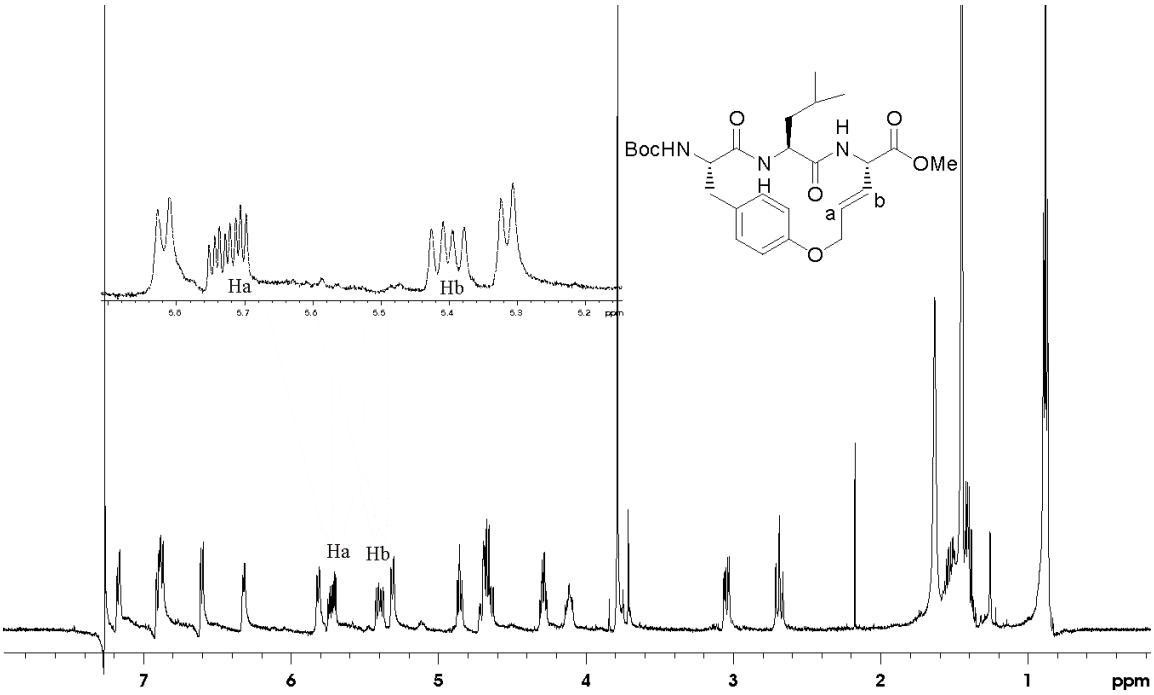


Figure 2.6. ^{1}H NMR spectrum of macrocycle **2.1**.

The microwave assisted RCM of **2.5** (condition B, Table 2.2) resulted in an improved yield of macrocycle **2.1** (58%) after purification by chromatography. The addition of chlorodicyclohexylborane to the reaction mixture of **2.5**, in this case, gave no significant improvement in yield (see conditions C in Table 2.2). We suggest that the carbene intermediate could form a particularly stable five membered ring chelate in this case (see Figure 2.7). The addition of chlorodicyclohexylborane might not disrupt.

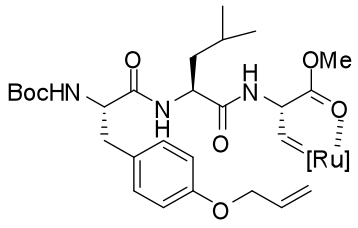


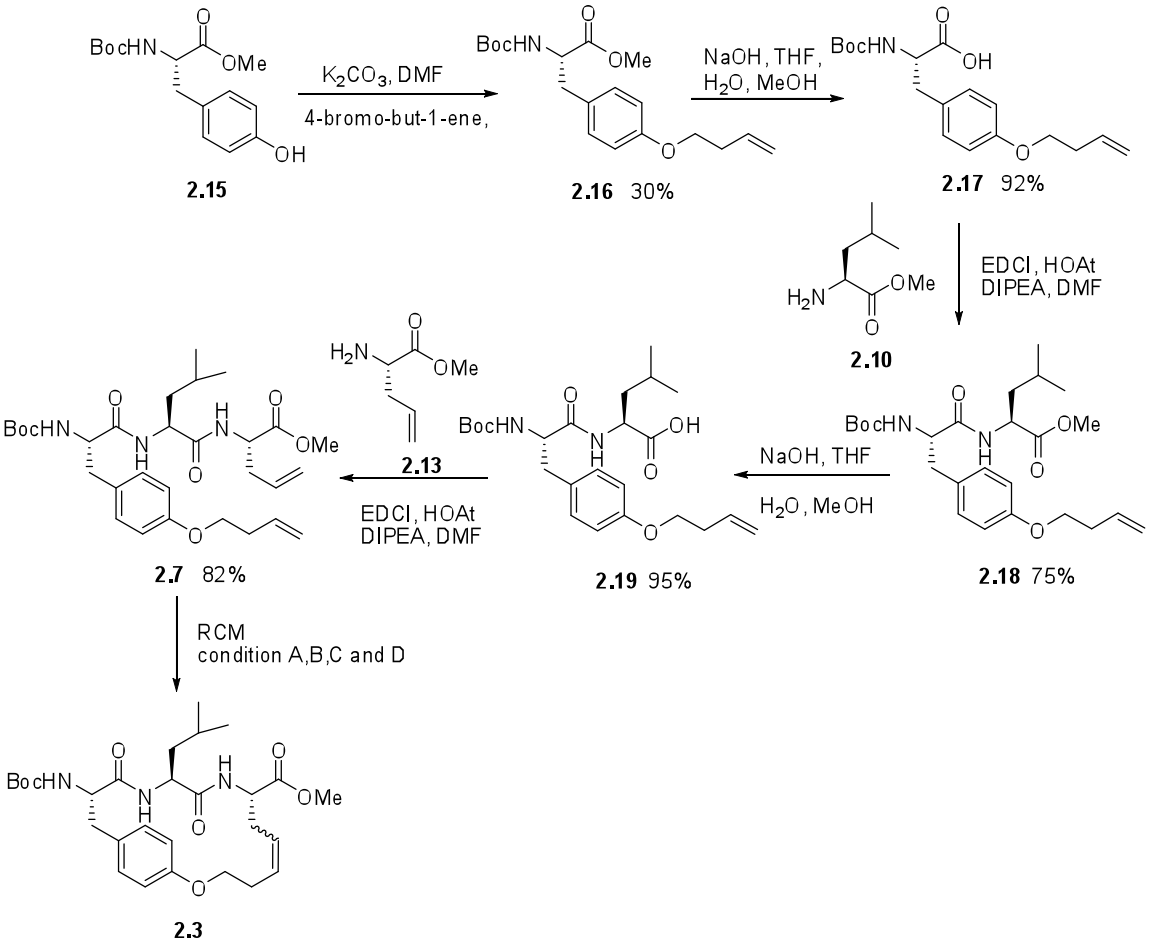
Figure 2.7. Structure of a five-membered chelate.

The addition of chlorodicyclohexylborane under microwave irradiation (condition D in Table 2.2) similarly did not improve the yield.

2.4: Synthesis of 18-membered macrocycle 2.3 *via* RCM under conditions A-D

The macrocycle **2.3** was prepared as shown in Scheme 2.4. Compound **2.19** was provided by Dr Steve Aitkin. **2.19** had been made from the commercially available *N*-Boc-Tyr-OMe **2.15**, *via* reaction with 4-bromobut-1-ene to give the homoallylic ether **2.16** in 30% yield. Hydrolysis of the methyl ester, under basic conditions, gave the carboxylic acid **2.17**, which was coupled with Leu-OMe **2.10** in the presence of EDCI/HOAt to give the dipeptide **2.18**. Hydrolysis of the ester then gave **2.19** in almost a quantitative yield.

Diene **2.7** was prepared by me by coupling of **2.19** with (*S*)-allyl-Gly-OMe **2.13**. Subsequent RCM was examined under varying conditions as shown in Table 2.3.



Scheme 2.4. Synthesis of 18-membered alkene macrocycle **2.3** by RCM.

RCM of **2.7** under the thermal conditions (condition A) gave macrocycle **2.3** in 51% (see Table 2.3). The molecular formula was confirmed by a parent ion in the mass spectra at m/z 532.3034. The ^{1}H NMR spectrum revealed two methyl ester peaks centered at 3.733 and 3.745 ppm in a ratio of 1.3:1. These were assigned to the *E* and *Z* isomers respectively (Figure 2.8). Two multiplets centered at 5.47 and 4.93 were also observed in a ratio of ca. 1:1.3 and assigned as alkene Ha of the *Z* (dd, J = 10.6, 8.0 Hz) and *E* isomers (ddd, J = 15.0, 7.0, 5.3 Hz) respectively. The major compound was again assigned as the *E* isomer based on the large coupling constant (15 Hz).

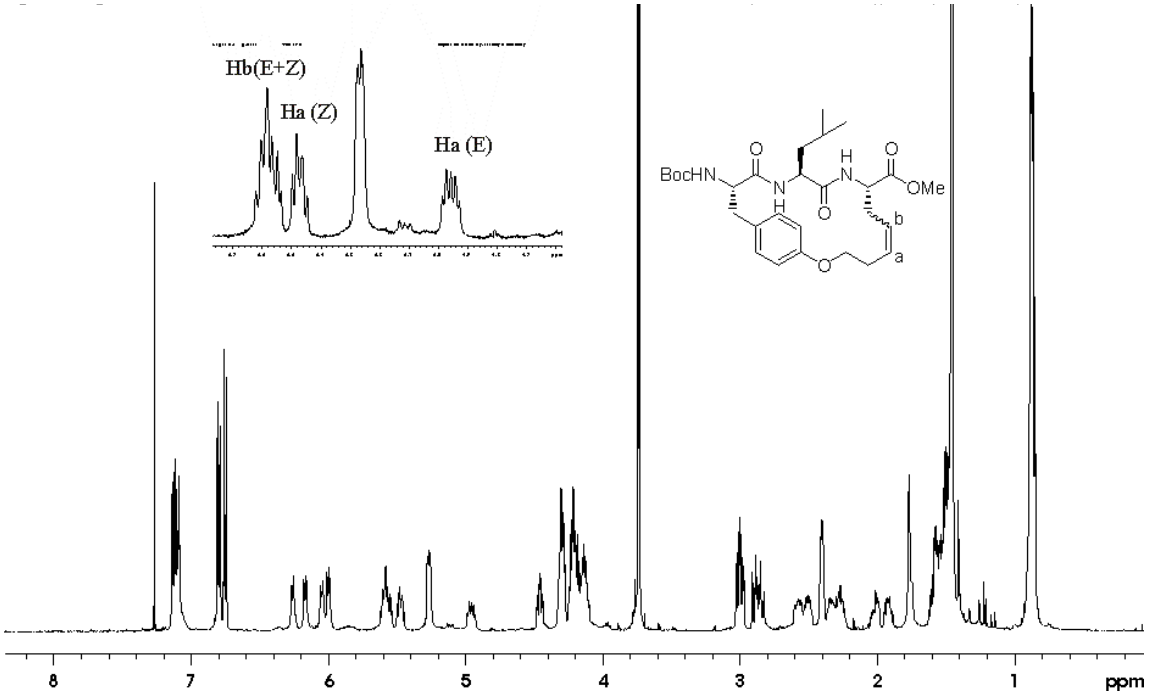


Figure 2.8. ^{1}H NMR spectrum of alkene macrocycle **2.3**.

RCM of **2.7** under microwave irradiation (condition B in Table 2.3) resulted in an improved yield of **2.3** (58%), with an *E*/*Z* isomers ratio of 1.7:1. A strikingly improved yield (74%) was observed under thermal reflux RCM conditions with the inclusion of a 10 mol% of chlorodicyclohexylborane (condition C, Table 2.3). The addition of chlorodicyclohexylborane and microwave irradiation (condition D) resulted in further improvement and a near quantitative yield of **2.3** (96%). The inclusion of chlorodicyclohexylborane, under both thermal and microwave conditions, increased the yield of macrocyclisation such that these conditions are considered best for a scaled up synthesis of **2.3**.

Table 2.3. RCM of diene **2.7** under different conditions

Diene	Condition [a]	Product	Ratio[b]	Yield [c]
2.7	A	**2.3**	1.3(*E*):1(*Z*)	51%
	B	**2.3**	1.7(*E*):1(*Z*)	58%
	C	**2.3**	1.9(*E*):1(*Z*)	74%
	D	**2.3**	1.8(*E*):1(*Z*)	96%

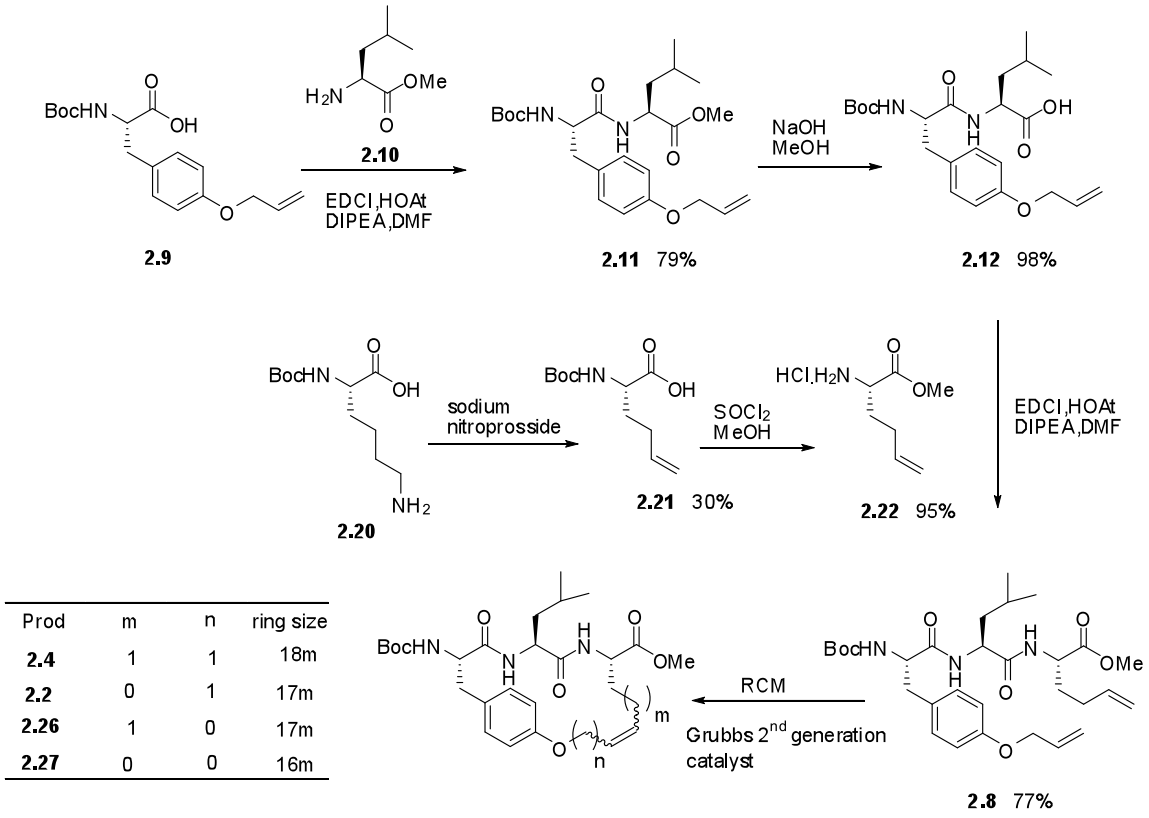
[a] All reactions performed in 1,1,2 TCE with 3 separate additions of 10 mol % Grubbs 2nd generation catalyst. Condition A: Thermal reflux 18 h. Condition B: Microwave reflux 1 h. Condition C: Thermal reflux with borane-based Lewis acid 18 h. Conditions D: Microwave reflux with Lewis acid 1 h. [b] RCM metathesis result in mixtures of geometric isomers. [c] Isolated yields following column chromatography.

2.5: Synthesis of 18-membered macrocycle 2.4 *via* RCM under conditions A-D

The required diene **2.8** was prepared as shown in Scheme 2.5. Coupling of commercially available *N*-Boc-Tyr (*O*-allyl) **2.9** with Leu-OMe **2.10**, in the presence of EDCI and HOAt, gave dipeptide **2.11** in 79% yield. Hydrolysis of the constituent methyl ester, under basic conditions, gave the carboxylic acid **2.12** in almost a quantitative yield.

The other key starting material **2.22** was prepared from *N*-Boc-Lys-OH **2.20**. Diazotization of **2.20** with sodium nitroprusside, under basic aqueous conditions (pH 9), gave homoallyl substituted glycine derivative **2.21** in 30% yield.[11] The amino acid derivative **2.21** was then treated with thionyl chloride in methanol to give **2.22** as the hydrochloride salt in nearly quantitative yield.

Coupling of **2.12** with the homoallyllic hydrochloride salt glycine **2.22**, in the presence of EDCI/HOAt, gave diene **2.8** in 77% yield. A mass spectrum confirmed the expected molecular formula with a parent ion at m/z 504.2727. The ^{1}H NMR spectrum showed three resonances at 4.52 ppm, 4.50 ppm and 4.38 ppm corresponding to α-protons of leucine, homoallylglycine and tyrosine, respectively.



Prod	m	n	ring size
2.4	1	1	18m
2.2	0	1	17m
2.26	1	0	17m
2.27	0	0	16m

Scheme 2.5. Synthesis of 18-membered alkene macrocycle **2.4** by RCM.

RCM of diene **2.8** (500 mg) using Grubbs 2nd generation catalyst under thermal conditions (A), resulted in some ring contraction to give a mixture of 18- and 17-membered macrocycles **2.4** and **2.2** (102 mg) as demonstrated by the presence of two parent ions in the mass spectrum: 532.28 and 518.27 m/z respectively (see Tables 2.4). The product mixture could not be separated by column chromatography. The ^{1}H NMR spectrum of the mixture showed the presence of three methyl ester signals centered at 3.732 ppm, 3.747 ppm and 3.756 ppm in the ratio of 2.7:1:1(see Tables 2.5).

Table 2.4. RCM of diene **2.8** under different conditions

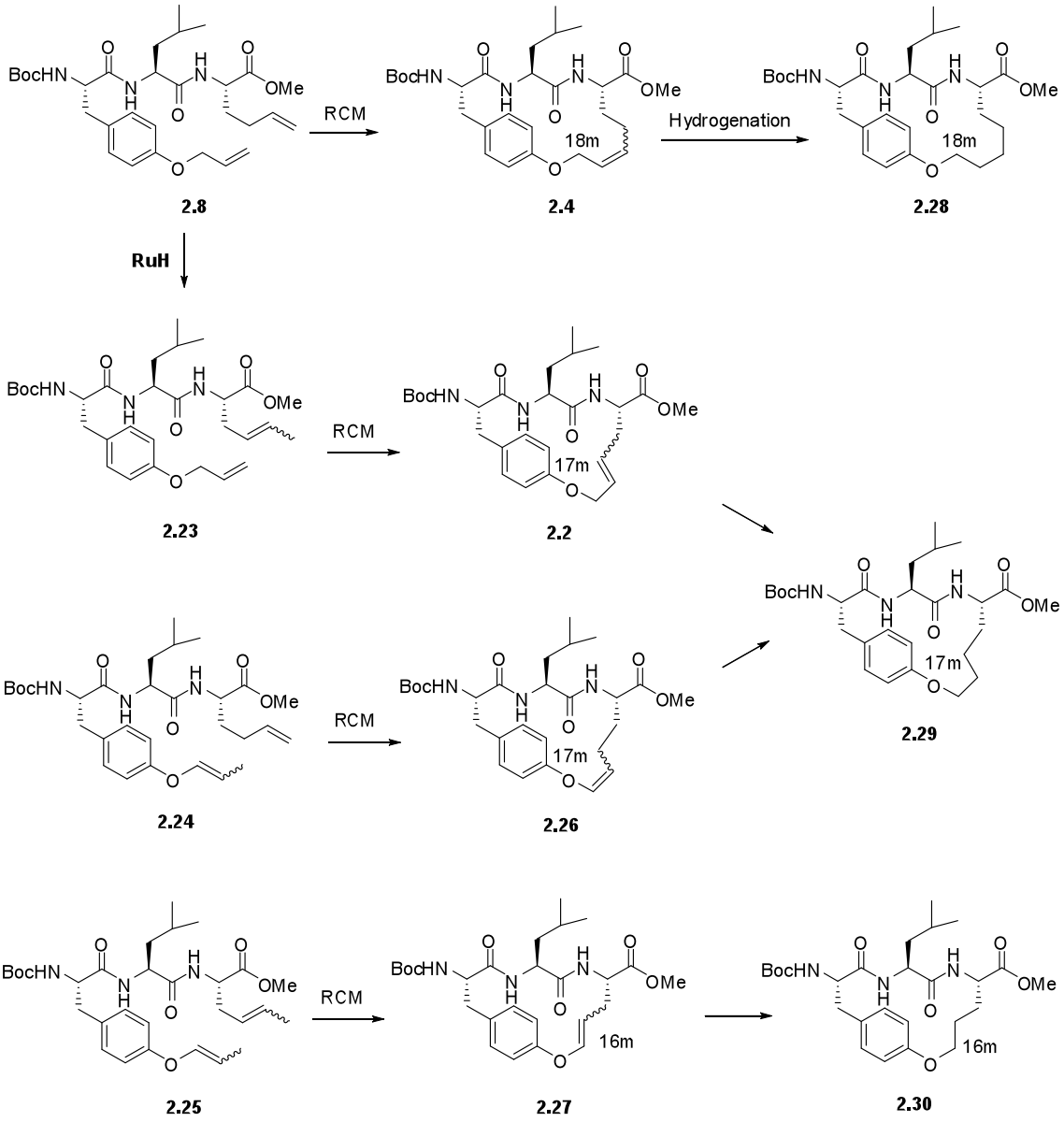
Diene **2.8**	Condition [a]	Product[b]	Amount [c]
500mg	A	18- and 17- membered	102 mg
500mg	B	18-, 17- and 16- membered	169 mg
500mg	C	18- and 17- membered	240 mg
500mg	D	18-, 17- and 16- membered	191 mg

[a] All reactions performed in 1,1,2 TCE with 3 separate additions of 10 mol % Grubbs 2nd generation catalyst. Condition A: Thermal reflux 18 h. Condition B: Microwave reflux 1 h. Condition C: Thermal reflux with chlorodicyclohexylborane 18 h. Conditions D: Microwave reflux with Lewis acid 1 h. [b] Multiple macrocyclic products were formed. [c] Amount following column chromatography.

The formation of the 17-membered ring compound **2.2** was consistent with a methyl ester resonance at 3.756 ppm which was identical to an authentic sample as prepared in Scheme 2.2. The resonances centered at 3.732 ppm and 3.747 ppm were observed in ratio of 2.7:1 by ^{1}H NMR and were assigned to the *E*/*Z* isomers of the 18-membered macrocycle **2.4**.

This ring contracted product **2.2** likely results from double bond migration of the homoallyl substituent of diene **2.8** (Scheme 2.6). A ruthenium hydride species formed by degradation of Grubbs catalyst has been previously reported to give rise to migration of a terminal double bond.[12] The 17-membered macrocycle **2.2** forms by RCM of diene where the double bond migrated on the homoallyl substituent (**2.23**).

The alkene mixtures formed were then subjected to hydrogenation in the presence of palladium on carbon to give a mixture of saturated macrocycles **2.28** and **2.29** as confirmed by the molecular ions in mass spectrum at m/z 534.30 and 520.30, respectively (see Scheme 2.6).



Scheme 2.6. Proposed migration of double bonds in RCM, resulting in reduced ring size macrocycles

Microwave assisted RCM of **2.8** (condition B in Table 2.5) gave a mixture of 18-, 17- and 16-membered alkene macrocycles **2.4**, **2.2**, **2.26**, **2.27**, as confirmed by the appropriate molecular ions in the mass spectrum, 532.28, 518.27 and 504.28 m/z (Figure 2.9).

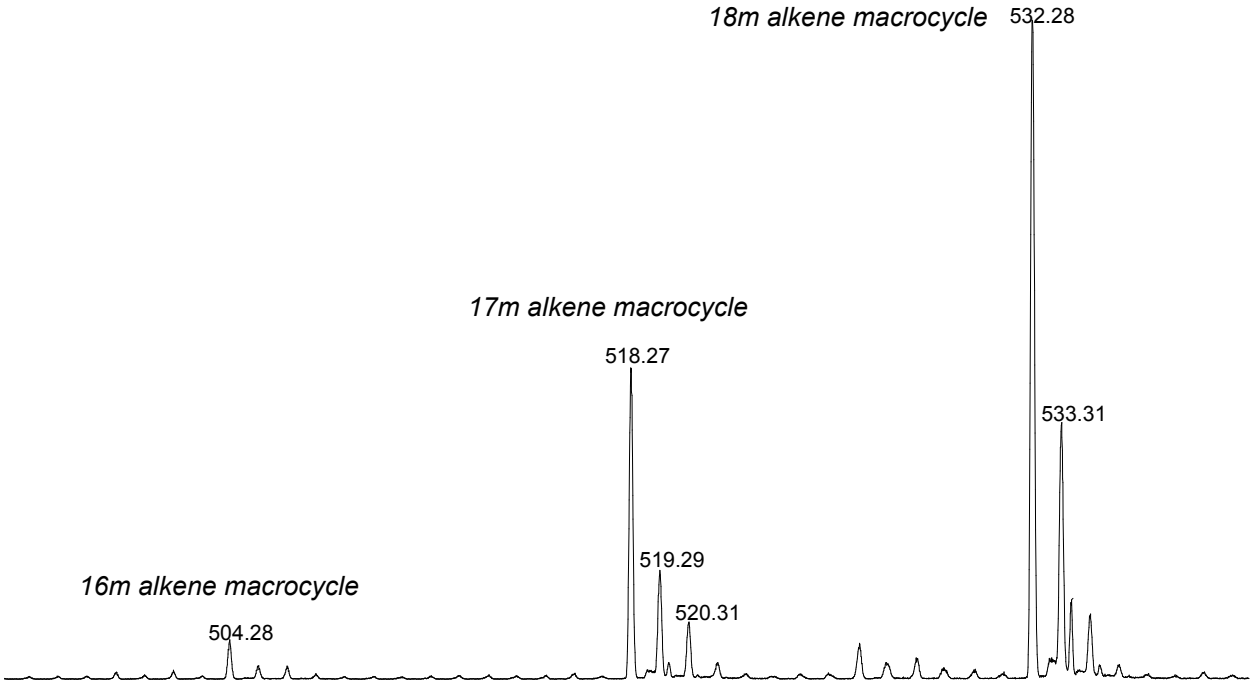


Figure 2.9. Mass spectrometry of alkene macrocycles resulting from double bond migration in the RCM of **2.8**.

The localized higher temperature associated with the microwave conditions results in greater double bond migration with a corresponding increase in 17- and 16-membered ring macrocycles. It has been reported that the extent of migration of a double bond depends on the metathesis conditions, particularly the reaction temperature.[13] The ^{1}H NMR of the mixture showed the presence of six methyl ester resonances centered at 3.779, 3.757, 3.745, 3.740, 3.732, 3.724 ppm in the ratio of 1.3: 12.1: 1.6: 1: 5.2: 7.3. Two signals centered at 3.732 and 3.745 ppm, in the ratio of 3.4:1, correspond to two of

the methyl ester signals formed under conditions A and correspond to the *E*/*Z* isomers of 18-membered macrocycle **2.4**. The signals at 3.757 and 3.779 ppm, observed in a ratio of 9:1, correspond to two methyl esters of the 17-membered ring macrocycle **2.2** as confirmed by comparison with the samples as prepared asScheme 2.2. A molecular ion at 504.28 m/z (see Figure 2.9) suggests the formation of a 16-membered ring compound **2.27** and therefore at least one of the remaining two methyl ester signals at 3.703 and 3.723 ppm are considered to be from the *E* or *Z* isomers of a 16-membered ring **2.27**.

Table 2.5. RCM of diene **2.8** under different conditions

diene	conditions[a]	products[b]	Ring size	OCH_3 (ppm)	isomer ratio[c]
2.8	A	**2.4**	18	3.732, 3.747	(E/Z) 2.7:1
		2.2	17	3.756	*E*
	B	**2.4**	18	3.732, 3.745	(E/Z) 3.4:1
		2.2	17	3.757, 3.779	9:1
		2.27	16	3.724 /3.740	*E/Z*
	C	**2.4**	18	3.732, 3.747	3.5:1
		2.2	17	3.757	*E*
	D	**2.4**	18	3.732, 3.744	3.4:1
		2.2	17	3.756, 3.778	9:1
		2.27	16	3.724/3.739	*E/Z*

[a] All reactions performed in 1,1,2 TCE with 3 separate additions of 10 mol % Grubbs 2nd generation catalyst. Condition A: Thermal reflux 18 h. Condition B: Microwave reflux 1 h. Condition C: Thermal reflux with borane-based Lewis acid 18 h. Conditions D: Microwave reflux with Lewis acid 1 h. [b] Multiple macrocyclic products were formed. [c] RCM result in mixtures of geometric isomers.

RCM with the addition of chlorodicyclohexylborane to the reaction mixture of **2.8** under both thermal reflux (condition C) and microwave condition (D) did not significantly improve the yield of macrocyclization (Table 2.4). The reaction of homoallyl group in **2.8** with ruthenium catalyst could result in a seven-membered chelate (see Figure 2.10) but the decreased stability of this chelate compared with the 6-membered chelate (Figure 2.4)

suggests that this chelate would unlikely interfere with cyclization. Thus these reactions were not affected by the addition of a Lewis acid.

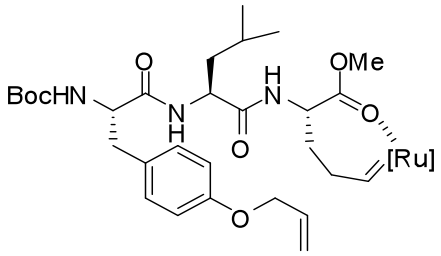


Figure 2.10. Structure of a seven-membered chelate.

In all cases, the alkene mixtures formed under each of the four conditions (A-D, Table 2.5) were separately subjected to hydrogenation to give saturated compounds as shown in Scheme 2.6. The alkene macrocycles prepared under conditions A and C gave a mixture of two compounds, as shown by the molecular ions in the mass spectrum at 534.30 and 520.30 m/z consistent with the formation of **2.28** and **2.29** (see Scheme 2.6). Hydrogenation of alkenes formed under condition B and D gave mixtures of three compounds exhibiting parent ions at 534.30, 520.30 and 506.28 m/z in the mass spectrum considered be from the 18, 17, 16-membered macrocycles **2.28, 2.29** and **2.30** (see Figure 2.11).

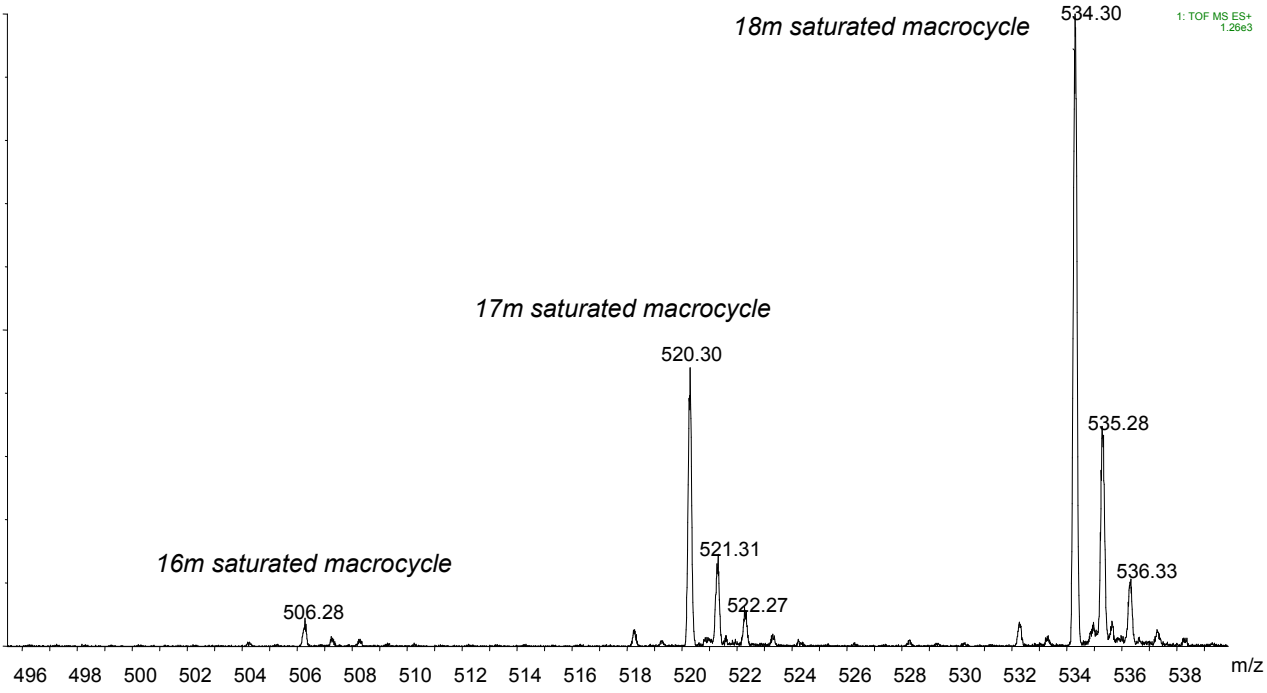
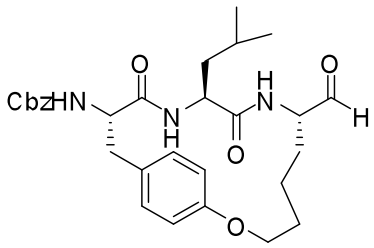


Figure 2.11. Mass spectroscopy of saturated macrocycles resulting from double bond migration in RCM of **2.8** under conditions B and D.

2.6: Synthesis of CAT811 via RCM under an optimum condition

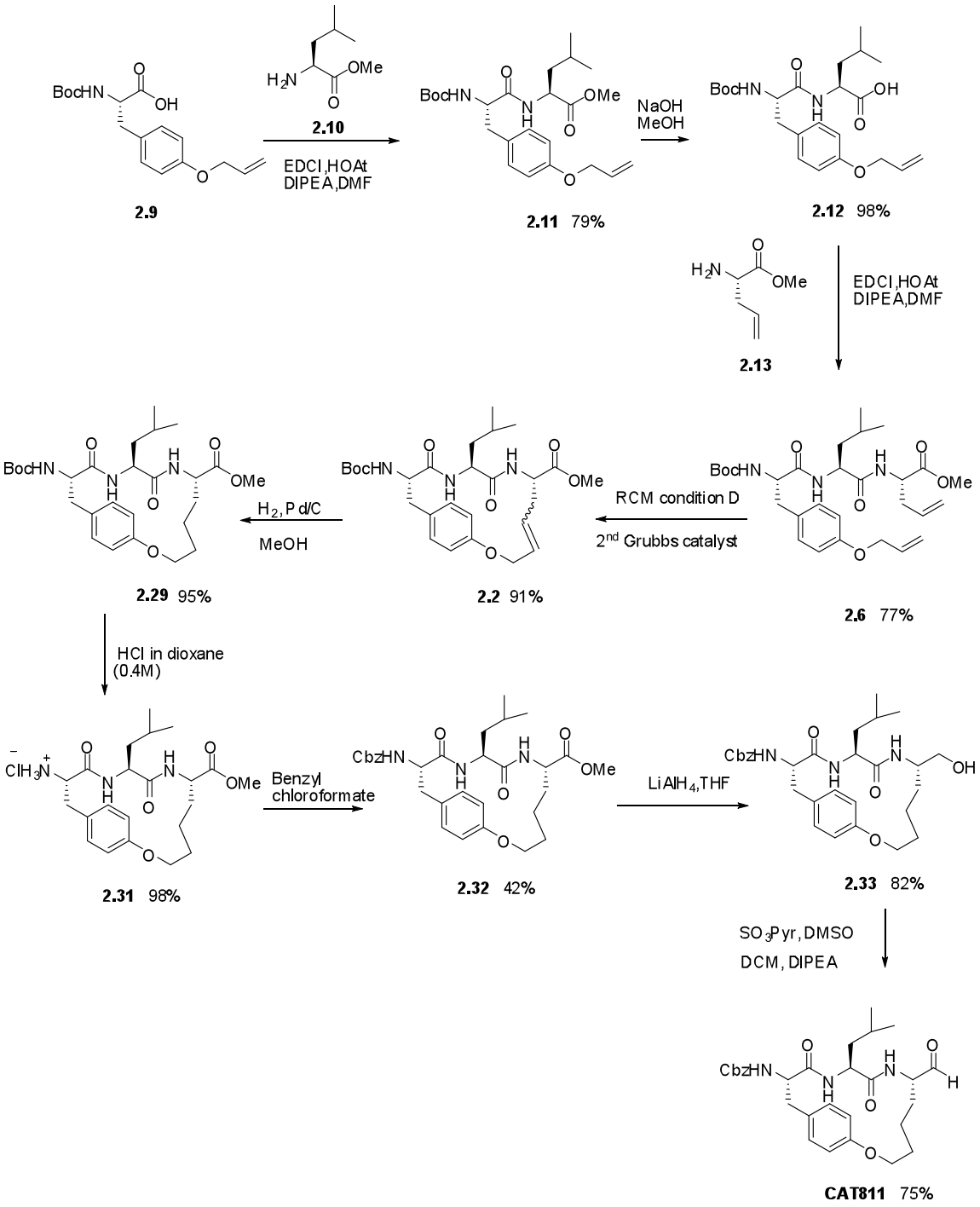
The published synthetic route to **CAT811** employed RCM under thermal reflux conditions as a key step. This reaction provided a modest yield of the product (22%). As discussed above (see section 2.2 and Table 2.1), the best RCM conditions developed in the current study involved the use of chlorodicyclohexylborane and microwave irradiation. A synthesis of **CAT811** was therefore carried out under these conditions (Scheme 2.7).



CAT811

EDCI catalysed coupling of commercially available **2.9** with **2.10** in the presence of HOAt gave **2.11** in 79% yield. Hydrolysis of ester **2.11**, under basic conditions, gave carboxylic acid **2.12** in nearly quantitative yield. An EDCI mediated coupling of **2.12** with **2.13** gave **2.6** in 77%. RCM of **2.6** was carried out, under the "best" conditions, to give the desired alkene macrocycle **2.2** in 91% yield, a 4-fold increase compared to the published thermal conditions (22%, see Table 2.1, condition D).

Reaction of **2.2** in a hydrogen atmosphere in the presence of palladium on carbon then gave the saturated macrocycle **2.29** in almost quantitative yield (95%). The Boc protecting group of compound **2.29** was removed by treatment of hydrochloride acid in dioxane (4 M) and the resulting hydrochloride salt **2.31** reacted with benzyl chloroformate to give Cbz-protected ester **2.32** in 42% yield. Reduction of **2.32** with lithium aluminium hydride in THF gave alcohol **2.33** (82%) which was oxidized with SO_3/Pyr and DMSO to give the aldehyde **CAT811** in 75% yield.



Scheme 2.7. Synthesis of **CAT811** by using the best RCM conditions.

2.7: X-ray analysis of 2.2 showing the (*E*)-configuration and *β*-strand conformation

A sample of an *E/Z* mixture of macrocycle **2.2** was recrystallised from methanol to give fine-plate like needles that had a ^{1}H NMR spectrum previously assigned to the *E*-isomer. Single crystal X-ray analysis of this material confirmed the (*E*)-isomer (Figure 2.12).[14] This is consistent with the coupling constant observed for the alkenic protons (J = 15.5 Hz) in the major alkene of the mixture.[6] The crystal structure also revealed that macrocycle **2.2** adopts a *β*-strand geometry for the peptide backbone as defined by P2 (Leu) Φ [C(4)-C(3)-N(2)-C(2)] and Ψ [N(2)-C(3)-C(4)-N(4)] torsion angles being within the ranges -160° < Φ < -100° and 90° < Ψ < 160° (see also Appendix C).

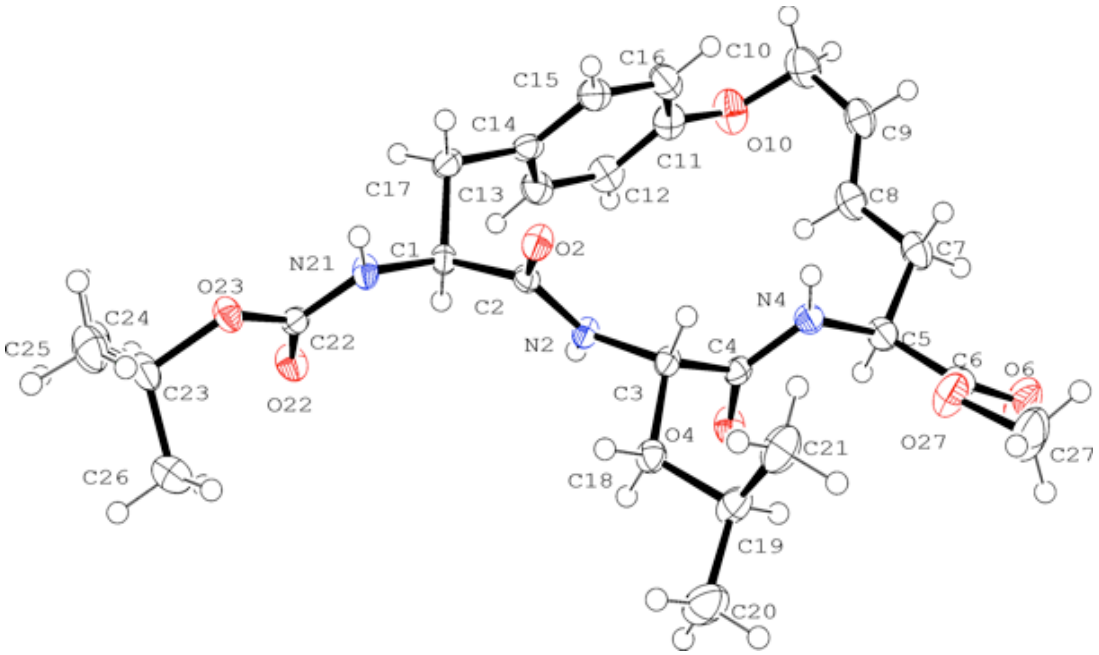


Figure 2.12. ORTEP diagram of the X-ray crystal structure of (*E*)-**2.2** showing a *β*-strand peptide backbone with Φ = -147.3° and Ψ = 119.7° with respect to the P2 (Leu) Φ and Ψ angles.

2.8: Conclusion

Factors that influence of efficiency of RCM of dienes **2.5-2.7** have been investigated. The addition of chlorodicyclohexylborane to the reaction mixture for the RCM of **2.6** and **2.7** results in an improved yield of the respective macrocyclic products **2.2** and **2.3**. This may be attributed to chlorodicyclohexylborane interfering with the formation of a 6-membered ring intramolecular ruthenium carbene chelate. However, the addition of chlorodicyclohexylborane did not improve the yield of RCM of diene **2.5**. In this case, the more stable 5-membered ruthenium carbene chelate is presumed not to be disrupted by the addition of Lewis acid. The addition of titanium (IV) isoproxide as an Lewis acid to RCM of **2.6** results in a lower yield of **2.2** compared to the use of chlorodicyclohexylborane.

Ring contraction was observed in the RCM reaction of diene **2.8** under reflux conditions to give the 17-membered ring macrocycle **2.2** in addition to the expected 18-membered macrocycle **2.4**. Reaction under microwave irradiation conditions gave mixtures of the 16-, 17- and 18-membered macrocycles. The ring contracted macrocycles are considered to result from double bond migration on either or both of homoallyl and allyl substituents of diene **2.8**.

The configuration of alkene in the macrocycles **2.1**, **2.2** and **2.3** was determined by ^{1}H NMR analysis. The alkene coupling constants for **2.1**, **2.2** and **2.3** (J = 15.5, 15.5 and 15.0 Hz, respectively) are consistent with the (*E*) configuration for the major isomer in each case. The configuration of the major alkene product (**2.4**) could not assigned as ring contraction gives a mixture of macrocycles.

A detailed X-ray crystallographic structural investigation was undertaken for macrocycle **2.2** revealing the (*E*) configuration and a *β*-strand conformation.

The best RCM reaction conditions we developed involved the use of chlorodicyclohexylborane and microwave irridiation. These conditions were used in a synthesis of our most potent calpain inhibitor **CAT811** and resulted in a 4-fold increase in yield.

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[14] The structures were solved by standard methods, and the atomic coordinates have been deposited with the Cambridge Structural Database.

Chapter 3: A new approach to the synthesis of CAT811 and analogue 3.21

3.1: Introduction

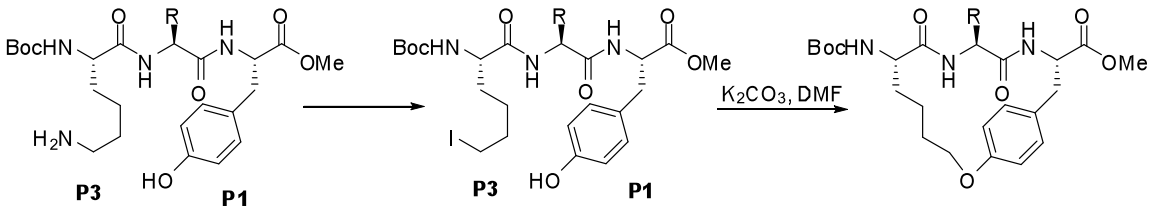
As discussed in Chapters 1 and 2 the 17-membered macrocyclic aldehyde **CAT811** is a potent inhibitor of calpain 2 (30 nM), and to a lesser extent calpain 1 (270 nM),[1] and it shows significant promise in the treatment of cataract. A large scale synthesis of **CAT811** was required to obtain material for *in vitro* or *in vivo* testing.[2]

The original synthesis of **CAT811** is based on a ring closing of diene **2.6** to give the alkene macrocycle **2.2**, which was converted by standard chemical methods into **CAT811** (see Scheme 2.7, Chapter 2). This synthetic route involves nine steps and occurs in an overall yield of 12% using the optimized conditions developed in Chapter 2. Several problems were expected on applying this strategy to a large scale synthesis. Ring closing metathesis is expensive as it requires the expensive and patented Grubbs second generation catalyst. The benzylation (step 7, Scheme 2.7) involves the use of benzylchloroformate which can result in environmental and safety problems. Moreover hydrogenation of **2.2** to **2.29** (step 5, Scheme 2.7) is normally conducted under 20 atmosphere pressure, which is not desirable for a larger scale laboratory synthesis. The hydrogenation apparatus available to us only achieves pressure to 500KP (ca 5 At). Finally, the original synthesis uses a flammable reducing agent $LiAlH_4$ for the reduction of the ester of **2.32** to the alcohol **2.33** (step 8). An alternative strategy was therefore required for a multi-gram synthesis of **CAT811**.

3.2: Application of an intramolecular nucleophilic substitution strategy for macrocyclisation

Farlie[3] has reported the preparation of 17-membered macrocyclic β-strand compounds **3.1a-c** (shown in Scheme 3.2) as templates for the development of HIV-1 protease inhibitors, using an intramolecular nucleophilic substitution strategy for ring closure. In

his method the primary amine in **3.28a-c** at P1 is converted into an iodide, as in **3.29a-c** (step 1) by diazotization of Boc-Lys with sodium nitroprusside under basic aqueous conditions to give the primary alcohol. This alcohol was converted to a bromide by treatment with triphenylphosphine (PPh_3) and tetrabromomethane (CBr_4) and the bromide converted to the iodide by refluxing with sodium iodide in acetone. The resulting tripeptide precursor **3.29a-c** undergoes intramolecular cyclisation (step 2) with the halide in P3 being displaced by the phenol nucleophile in P1 to give the macrocycles templates **3.1a-c**.



3.28 a: R=$CH(CH_3)_2$
b: R=C_4H_9
c: R=$CH_2OC_3H_7$

3.29 a: R=$CH(CH_3)_2$
b: R=C_4H_9
c: R=$CH_2OC_3H_7$

3.1 a: R=$CH(CH_3)_2$
b: R=C_4H_9
c: R=$CH_2OC_3H_7$

Scheme 3.2. Synthesis of macrocycles **3.1a-c** by intramolecular nucleophilic substitution.

An advantage of this intramolecular nucleophilic substitution strategy, over the ring closing metathesis route, is that it avoids the use of the expensive Grubbs reagent. Clemene Buisan, a French summer undergraduate student in our group at UC investigated a small scale synthesis of **CAT811** as shown in Scheme 3.3 using this methodology. This chapter describes the optimisation of this preliminary synthesis on a larger scale and the completion of the last step which was not undertaken. [4] We prepared 1.5 grams of **CAT811** by this route.

A retrosynthetic analysis of **CAT811** is shown in Figure 3.1 where cyclisation occurs by a reaction in which halide in P1 is displaced by the hydroxyl of the tyrosine residue in P3.[5] The corresponding full synthetic route is shown in Scheme 3.3.

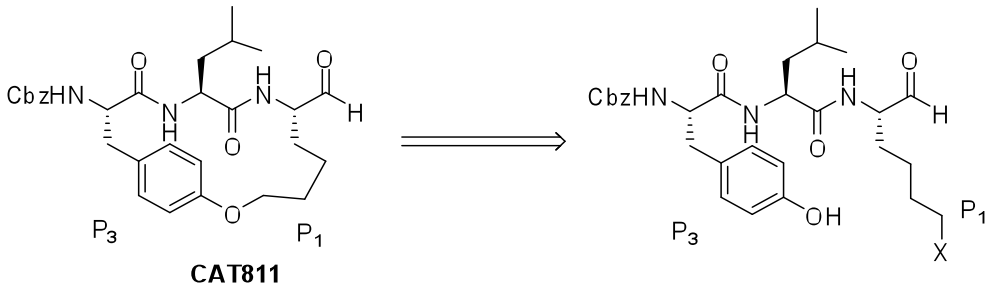
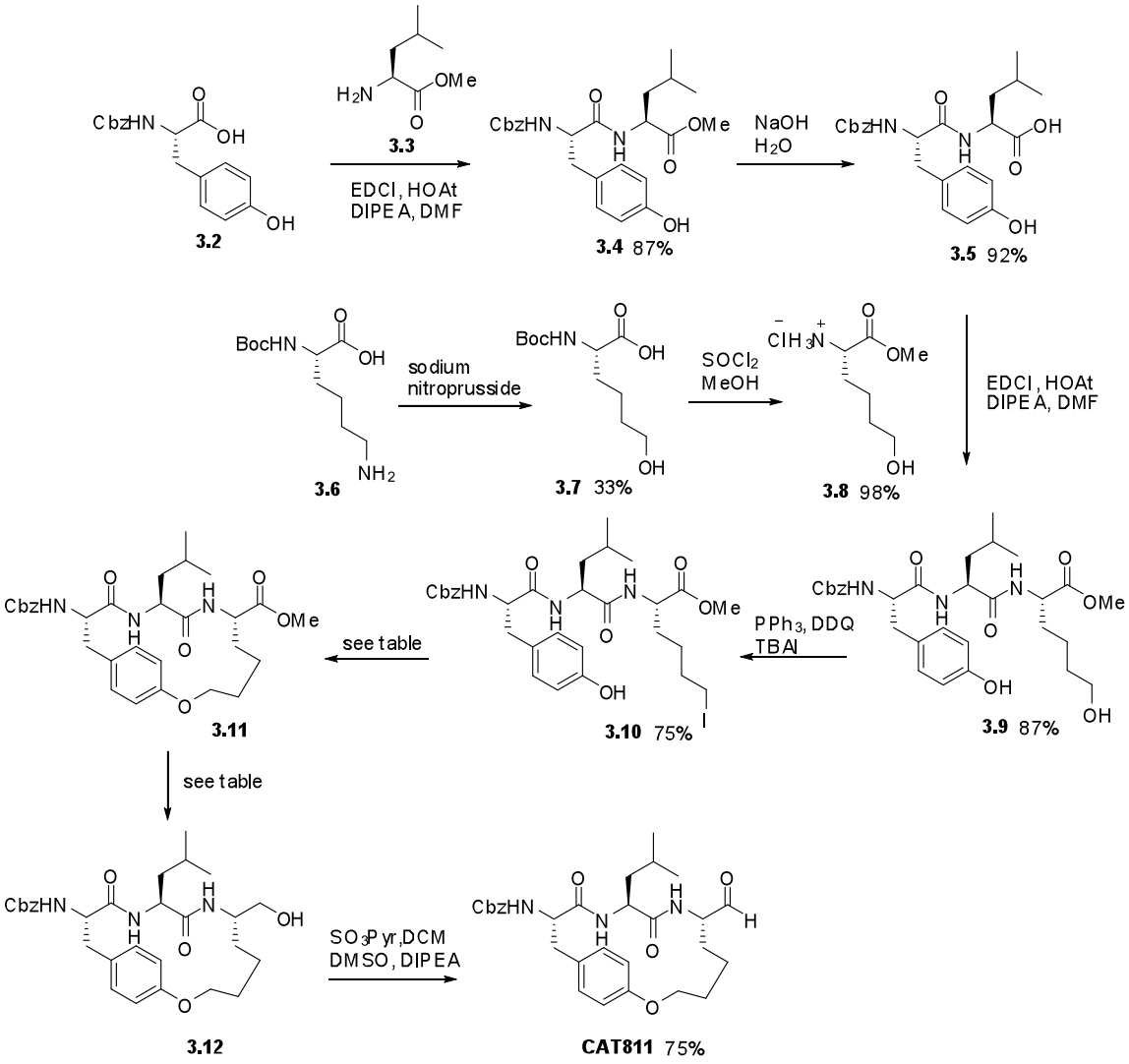


Figure 3.1. Retrosynthetic analysis of **CAT811**



Scheme 3.3. Synthesis of **CAT811** via intramolecular nucleophilic substitution

An EDCI[6] mediated coupling of *N*-Cbz-Tyr-OH **3.2** with Leu-OMe **3.3,** in the presence of HOAt and DIPEA, gave dipeptide **3.4** in 87%. The formation of **3.4** was confirmed by ^{1}H NMR analysis as shown in Figure 3.2, which revealed key resonances at 4.51 and 4.42 ppm corresponding to α-protons of tyrosine and leucine respectively.

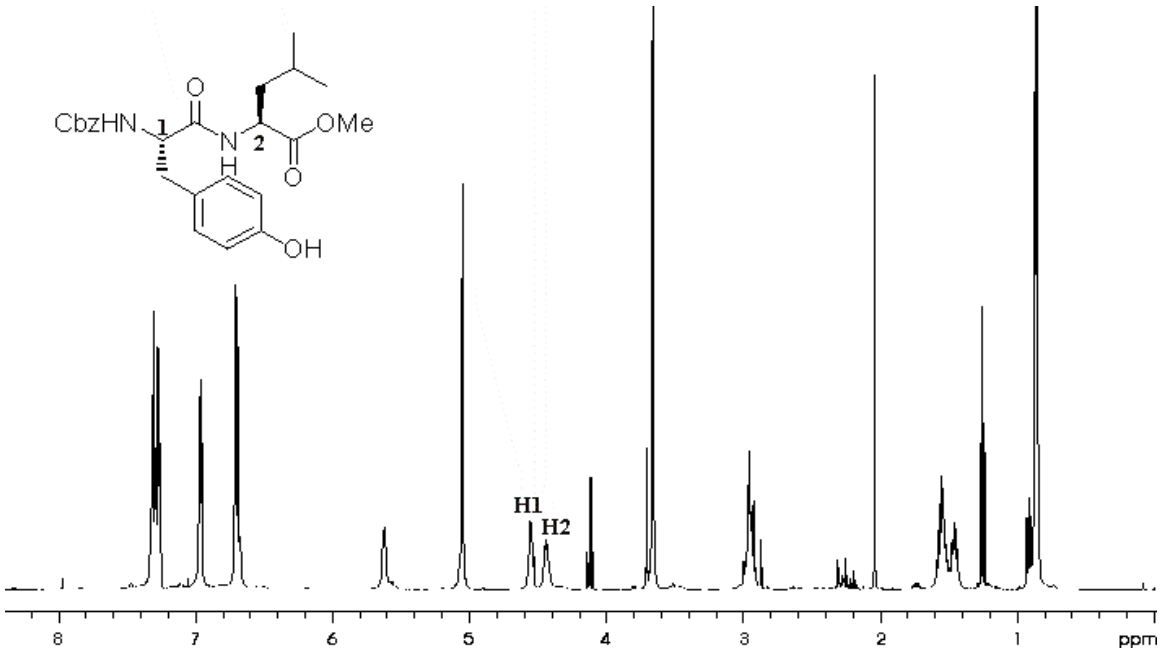


Figure 3.2. ^{1}H NMR spectrum of dipeptide **3.4** showing two α-protons.

Hydrolysis of the methyl ester of dipeptide **3.4** was carried out in aqueous sodium hydroxide solution to give **3.5**. The key compound **3.8** was prepared from commercially available Boc-Lys-OH **3.6** as shown in Scheme 3.3. In particular, diazotization of **3.6** with sodium nitroprusside, under a basic aqueous conditions (pH 9), gave **3.7** in 33% yield.[7] Simultaneous deprotection of N-Boc group and esterification of the carboxyl acid of **3.7**, by reaction with thionyl chloride in the presence of methanol, gave the hydrochoride salt **3.8**. Methanol reacts with thionyl chloride to give methoxy-sulfonyl chloride and releases hydrochloride acid. The acid sensitive *tert*-butoxycarbonyl group (Boc) of **3.7** is removed by acidolysis to give an ammonium salt, while the carboxyl group of **3.7** reacts with methyoxy sulfonyl chloride to form a carboxylic acid-sulfinic acid anhydride which facilitates nucleophilic substitution by methanol to give the desired

methyl ester. The resulting salt **3.8** was coupled with **3.5**, in the presence of EDCI and HOAt, to give the tripeptide **3.9** in 87% yield, the structure of which was confirmed by the presence of three α-protons centred at 4.41, 4.38 and 4.32 ppm in the ^{1}H NMR spectrum (Figure 3.3).

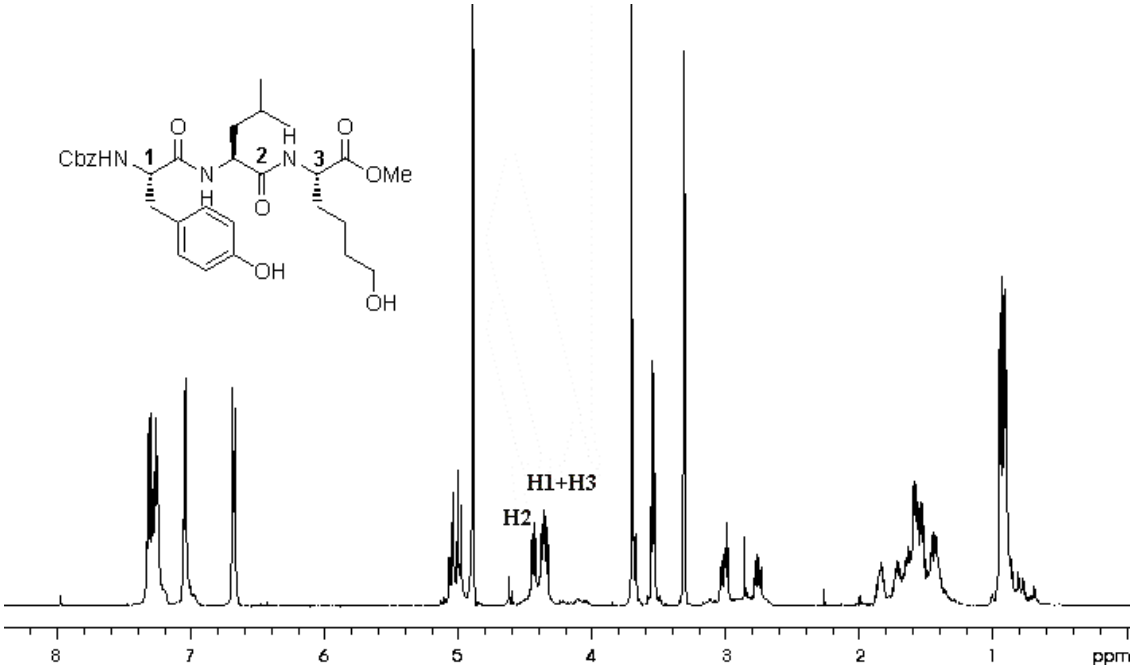
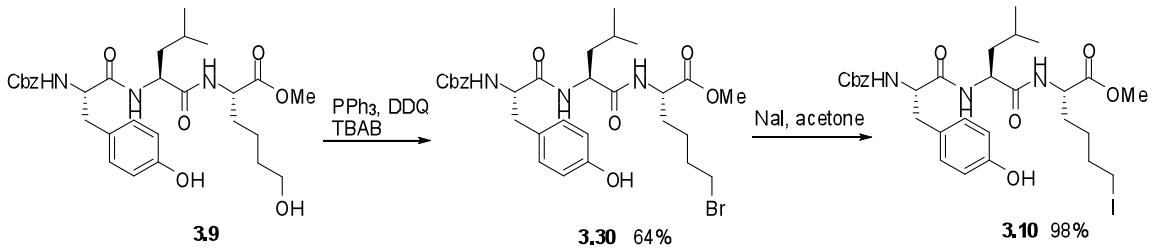


Figure 3.3. ^{1}H NMR spectrum of tripeptide Cbz-Tyr-Leu-Gly-OMe **3.4**.

The iodination of primary alcohol in **3.9** was next investigated. Iranpoor[8] has reported the use of triphenylphosphine (PPh_3), 2, 3-dichloro-5, 6-dicyano-1,4-benzoquinone (DDQ) and tetrabutyl ammonium halides for the conversion of alcohols, thiols and selenols into alkyl halides. The main advantage of this method is that it can be applied to the preparation of an alkyl halide from alcohols that contain other reactive functional groups, including carbon-carbon double bonds, carbonyl, amino, ethereal bonds. The use of a halide as a quaternary ammonium salt is safer and more convenient on a large scale synthesis relative to the use of molecular halogen.[9] Moreover this method shows high selectivity for conversion of a primary alcohol into the corresponding alkyl iodide. For example, the conversion of phenol to the corresponding halide is as expected not observed under these conditions. This method allows for the conversion of the primary

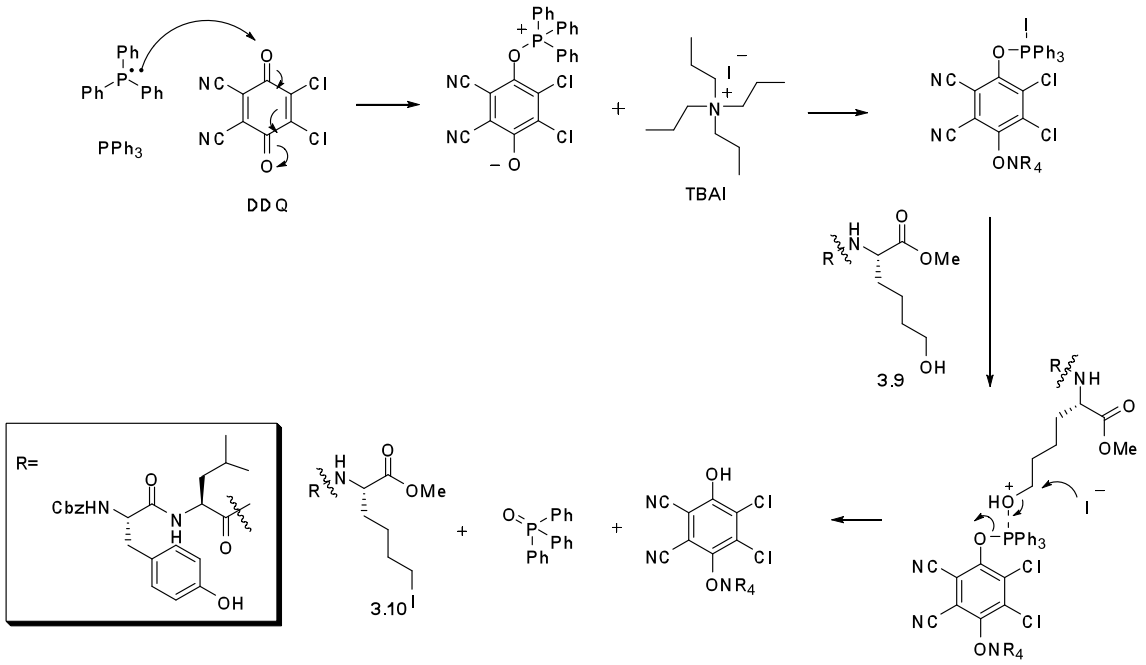
alcohol in **3.9** into the corresponding iodide (**3.10**) in the presence of phenol group of tyrosine.



3.9 **3.30** 64% **3.10** 98%

Scheme 3.4. Iodination of **3.9** to **3.10** *via* Iranpoor's method.

It proved more efficient to iodinate alcohol **3.9** directly using tetrabutylammonium iodide (TBAI), DDQ, and PPh_3. The mechanism proposed for the formation of **3.10** is shown in Scheme 3.5. The triphenylphosphine oxide by-product is separated from the iodide by recrystallisation from ethyl acetate and pentane.



Scheme 3.5. Proposed mechanism of iodination of primary alcohol **3.9** to give alkyl iodide **3.10**.

Macrocyclisation of **3.10** was investigated by nucleophilic displacement of the iodide by the hydroxyl of the phenol group of the tyrosine. Macrocyclisation was first attempted on a small scale (100 mg) by treatment of **3.10** with the amine base DIPEA in DCM (Table 3.1). However, only starting material was isolated after 16 h. The use of K_2CO_3 in toluene proved effective and gave macrocyclic product in 64% yield after chromatography. The use of K_2CO_3 in DMF or acetonitrile gave the somewhat improved yield of 67% and 68%, respectively. The best yield obtained was for reaction using K_2CO_3 in the presence of a catalytic quantity of Cs_2CO_3 (Table 3.1). A large scale reaction (> 5 g) under these conditions gave the desired macrocycle **3.11** in 70% yield after purification by recrystallisation from ethyl acetate and pentane.

Table 3.1. Conditions for the macrocyclization of **3.10**

Base	Solvent	Temperature/Time	Yield
DIPEA	DCM	Reflux/16 h	0%
K_2CO_3	Toluene	Reflux/16 h	64%
K_2CO_3	DMF	Reflux/16 h	67%
K_2CO_3	MeCN	Reflux/16 h	68%
K_2CO_3/Cs_2CO_3	MeCN	Reflux/16 h	70%

The final steps in the synthesis of **CAT811** (Scheme 3.3) involve the reduction of the C-terminal methyl ester of **3.11** to the alcohol **3.12**, followed by oxidation to give the desired aldehyde **3.1**. $LiAlH_4$ was used for this on a small scale, see Scheme 2.7.[10] However, $LiAlH_4$ reacts violently with water and the solvent can readily catch fire in the presence of moisture thereby limiting its use in large scale synthesis. $NaBH_4$ is easy to handle and its reducing power towards esters can be enhanced with the addition of metal salts, for example $AlCl_3$, $ZnCl_2$ and LiCl.[11]

Reduction of ester **3.11** was therefore carried out using $NaBH_4$ in the presence of various salts including $AlCl_3$, $ZnCl_2$ and LiCl to give the desired alcohol **3.12** in isolated yields of 9%, 36% and 90%, respectively. $NaBH_4$ reacts with LiCl to give $LiBH_4$ which allows for the reduction of ester. The reaction conditions were further optimised by directly using

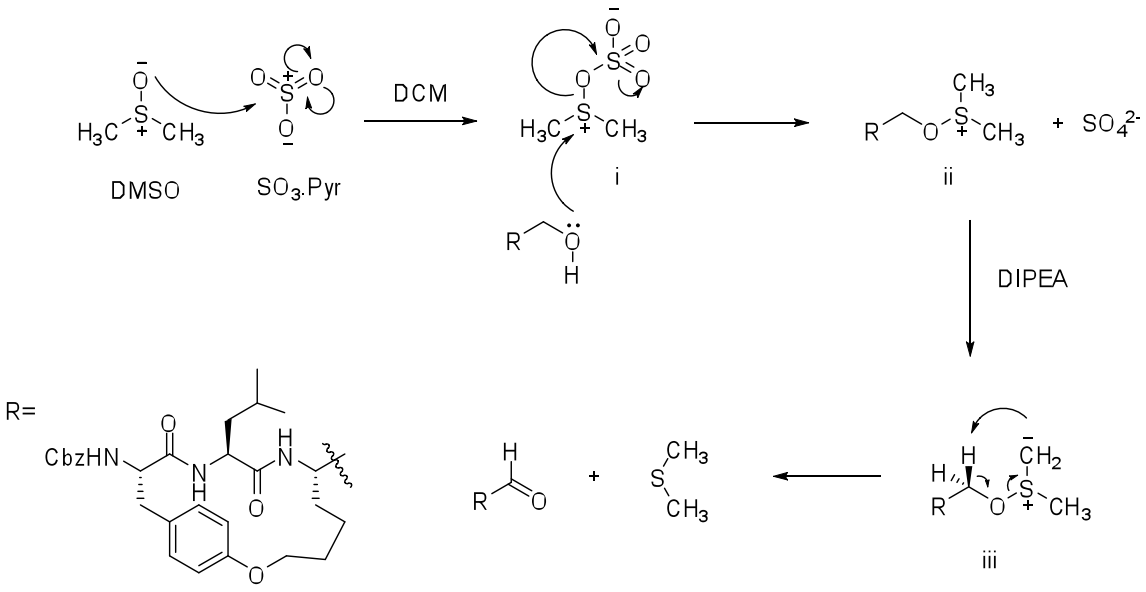
$LiBH_4$ in THF and this gave an improvement in the yield after recrystallisation from ethyl acetate of **3.12** (97%, see entry 4 in Table 3.2).

It has also been reported[12] that the Lewis acid $B(OMe)_3$ can catalyse the reduction of an ester by $LiBH_4$. The borane-based Lewis acid $B(OMe)_3$ is thought to function by coordination with the carbonyl oxygen to increase the electron deficiency of the carbonyl carbon thereby facilitating attack by borohydride. The use of $LiBH_4$ with $B(OMe)_3$ gave a somewhat improved yield of **12** (98%, see entry 5 in Table 3.2).

Table 3.2. Varying conditions for the reduction of **3.11**

Entry	Reducing agent	Solvent	Additive	Yield
1	$NaBH_4$	THF	$AlCl_3$	9%
2	$NaBH_4$	THF	$ZnCl_2$	36%
3	$NaBH_4$	THF	LiCl	90%
4	$LiBH_4$	THF	-	97%
5	$LiBH_4$	THF	$(MeO)_3B$	98%

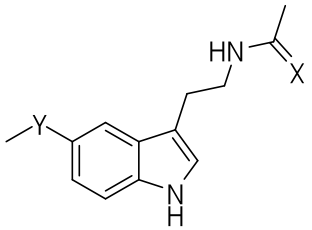
The alcohol **3.16** was then oxidized to **CAT811** (75%) using dimethyl sulfoxide (DMSO) in the presence of SO_3·pyridine complex.[13] DMSO is first activated by the electrophlic SO_3.Pyr to form a sulfonium species and the activated DMSO functions as an oxidizing reagent for oxidation of a primary alcohol to an aldehyde (Scheme 3.6). The sulfonium species contains a good leaving group linked to the electrophilic sulfur atom. The leaving group is displaced by alcohol to give an alkoxydimethylsulfonium salt which is followed by deprotonation by DIPEA to form the sulfur ylide. Intramolecular elimination leads to the formation of aldehyde and dimethyl sulfide.



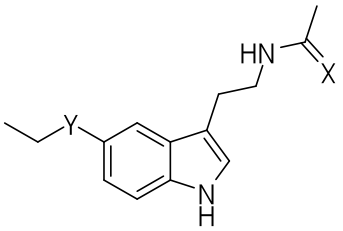
Scheme 3.6. DMSO oxidation with sulphur trioxide pyridine as an activating reagent.

3.3: Attempted synthesis of the related 19-membered Tyr-Leu-Ser based macrocycle 3.21

Oxygen and sulfur are isosteric having identical outer shells of electrons resulting in some chemical and physical similarity.[14] Isosteres are commonly used in drug design to vary the characteristics of molecules including size, polarity, electronic distribution and H-bonding characteristics. For example, Davies[15] reported that the sulfur and oxygen isosteres can affect the binding affinity and biological activity of melatonin **3.22** and N-acetyl 5-ethoxytryptamine **3.23** against human and amphibian melatonin receptors. The replacement of the oxygen atoms in **3.22** and **3.23** with sulfur gives analogues **3.24-3.27** which show decreased binding affinity and reduced potential on biological assay. For example, introducing sulfur in place of the amide oxygen (compound **3.24**) and methoxy oxygen (compound **3.25**) results in ca 2.5-fold and 35-fold reduction in bonding affinity to human melatonin receptors, respectively.

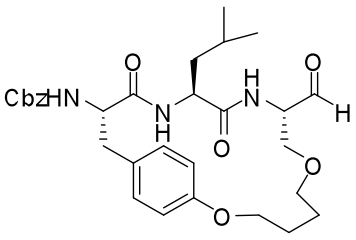


3.22 X=O, Y=O
3.24 X=S, Y=O
3.25 X=O, Y=S

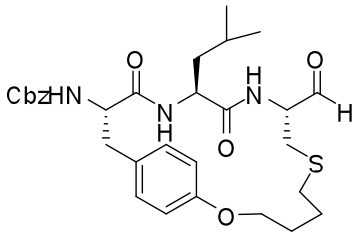


3.23 X=O, Y=O
3.26 X=S, Y=O
3.27 X=O, Y=S

A preparation of 19-membered macrocycle **3.21** was attempted using an analogous procedure to that discussed in the previous section for the preparation of **CAT811**. This derivative is an analogue of the known macrocycle **1.23a**[1], with the sulfur replaced by oxygen at the P1 position. The 19-membered macrocycle **1.23a** is reported to have modest activity against both calpain 1 (IC_{50} = 3.15 μM) and calpain 2 (IC_{50} = 1.01 μM) (see 1.4.2, Chapter 1).

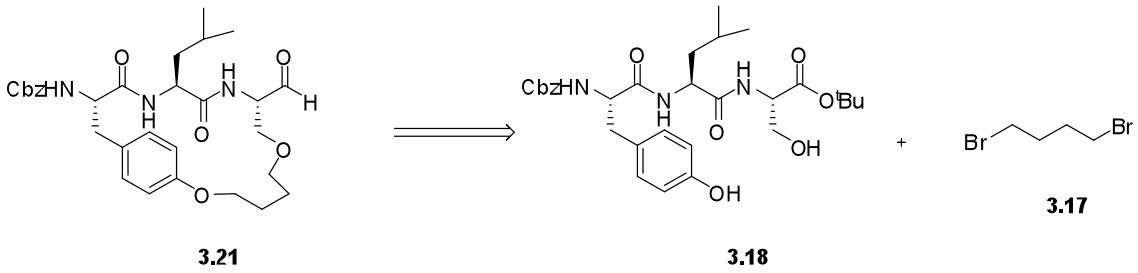


3.21



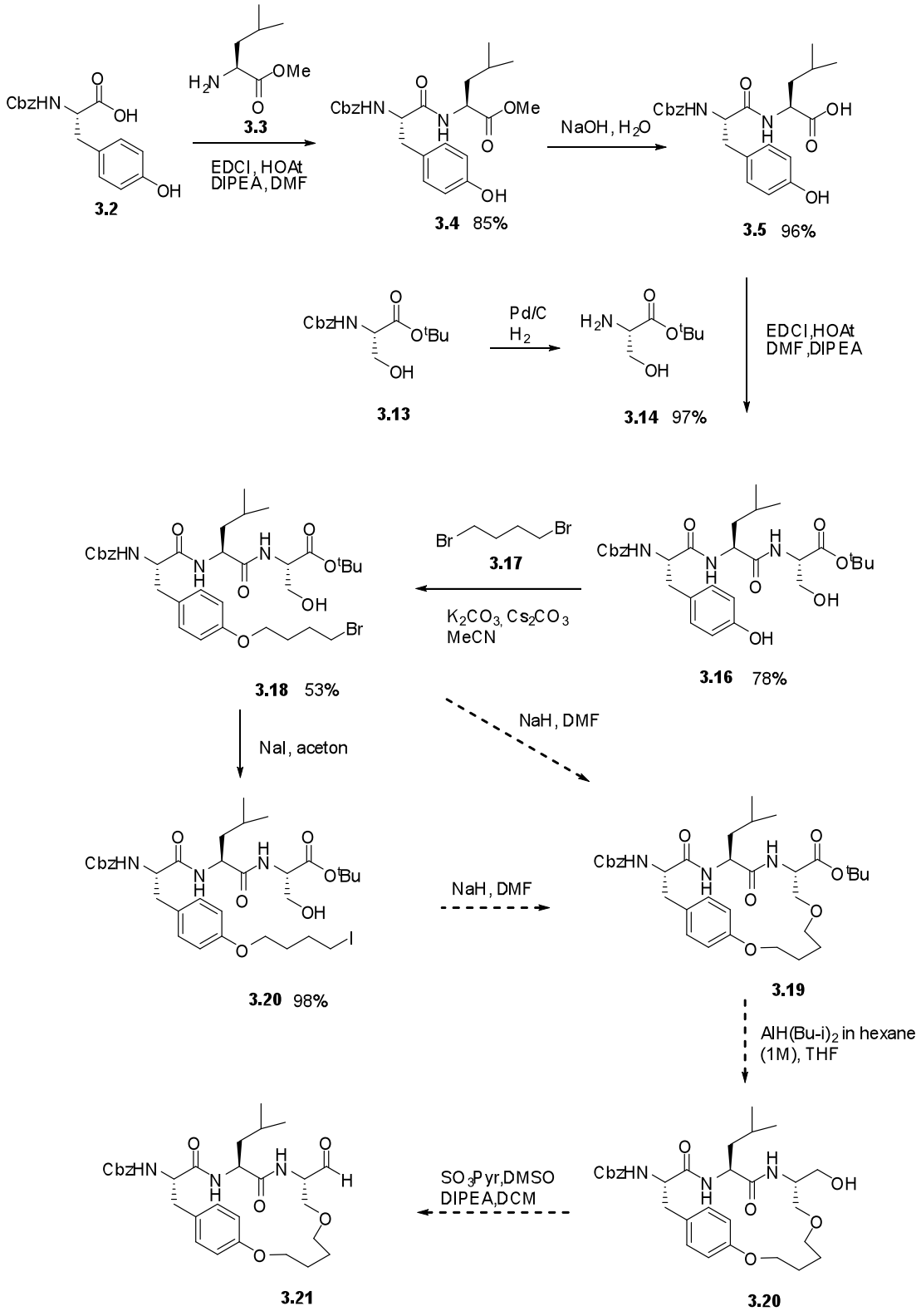
1.23a

The intramolecular nucleophilic substitution methodology developed for the synthesis of the 17-membered macrocycle **CAT811**, was therefore investigated in our attempted synthesis of the 19-membered macrocycle **3.21**. A retrosynthetic analysis of **3.21** (Scheme 3.7) suggests that macrocyclisation would be achieved by sequentially nucleophilic substitutions of 1,4-dibromobutane **3.17** by the phenol of tyrosine and the serine hydroxyl group of **3.18**. The phenol is more acidic and hence alkylation of the phenol of tyrosine with the appropriate alkyl halide would be expected to occur in the presence of K_2CO_3 and Cs_2CO_3 (see Table 3.1). A stronger base would be required to deprotonate the serine hydroxyl before reaction with dibromobutane **3.17**. The attempted synthesis of **3.21** was shown in Scheme 3.8.



Scheme 3.7. Retrosynthetic analysis of macrocycle **3.21**.

A synthetic route to macrocycle **3.21** based on an intramolecular nucleophilic substitution reaction is shown as Scheme 3.8. The route commenced with the coupling of *N*-Cbz-Tyr-OH **3.2** and Leu-OMe **3.3** in the presence of EDCI/HOAt to give the dipeptide **3.4** in 85% yield. Hydrolysis of the methyl ester of **3.4**, under basic conditions, followed by coupling with Ser-O^{t}Bu **3.14** in the presence of EDCI and HOAt, gave the tripeptide **3.16** in 78% yield.



Scheme 3.8. Attempted synthesis of 19-membered serine based macrocycle **3.21**.

The formation of **3.16** was confirmed by a parent ion in a mass spectrum at 572.23 m/z and three α-protons at 4.45, 4.55 and 4.58 ppm in the ^{1}H NMR spectrum (see Figure 3.4). The intermediate **3.14** was prepared by hydrogenation of commercially available *N*-Cbz-Ser-O^{t}Bu **3.13** under a hydrogen atmosphere in the presence of palladium on carbon.

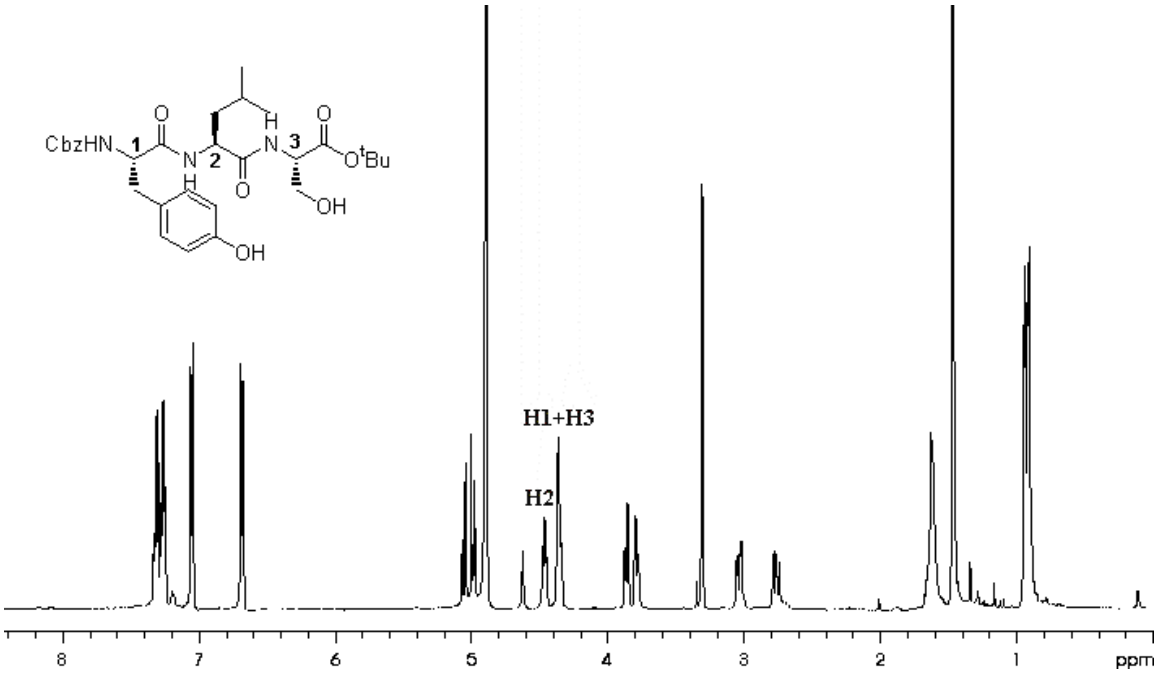


Figure 3.4. ^{1}H NMR spectrum of tripeptide **3.16** showing three α-protons.

Selective alkylation of the phenol of **3.16** was attempted by reaction with 1,4-dibromobutane **3.17** in the presence of K_2CO_3 and Cs_2CO_3. Alkylation gave the desired product **3.18** in 53% yield after purification by chromatography on silica. The molecular formula of **3.18** was confirmed by a parent ion in a mass spectrum at 728.2 m/z (MNa^+). The formation of **3.18** was confirmed by a Heteronuclear Multiple Bond Correlation (HMBC) experiments. As shown in Figure 3.5, H1 with a resonance at 3.90 ppm is coupled with C2 (two bond distant) and C3 (three bond distant) and in particular with one aromatic *C*-H carbon namely C5 (159.2 ppm) which is three bond distant. It is the coupling with C5 that defines that alkylation has occurred at phenol.

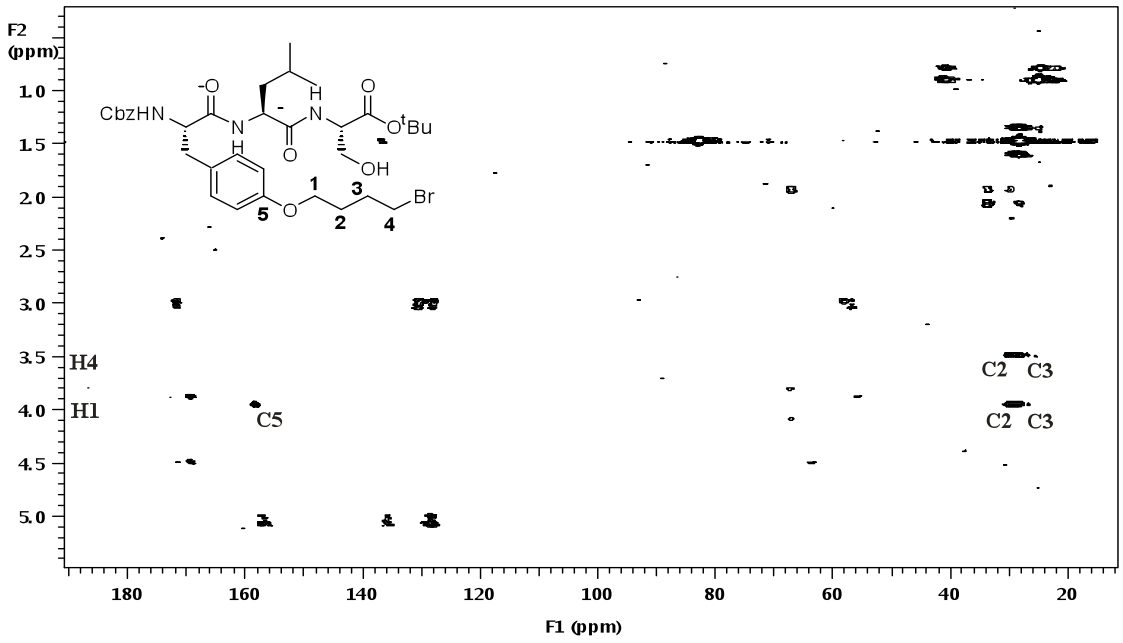
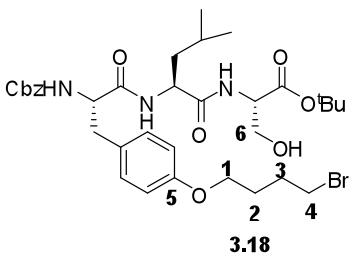
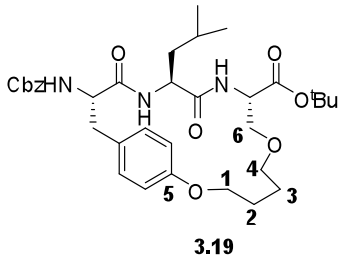


Figure 3.5. HMBC spectrum of compound **3.18**. Selected proton resonances H1 and H4 are labelled at the side of the spectrum and selected carbon resonances are labelled beside the coupled peak.

H4 at δ 3.45 ppm is only coupled with C2 and C3 confirming that substitution only occurred at the phenol (compound **3.18**). H4 would also have been coupled with the serine CH_2 group (namely C6) if alkylation had occurred on both the phenol and serine hydroxyl in forming the macrocycle **3.19**.



3.18



3.19

An intramolecular alkylation of serine hydroxyl of **3.18** was first attempted in the presence of NaH in anhydrous DMF. However, only starting material was isolated under these conditions as evidenced by the NMR spectrum of the crude reaction mixture.

Further attempts to prepare **3.19** involved conversion of alkyl bromide **3.18** to the alkyl iodide **3.20** as the iodide would be a better leaving group. The formation of **3.20** was confirmed by the ^{1}H NMR, which revealed a shift in the chemical shift of H4 protons from δ 3.42 ppm in bromide **3.18** to δ 3.28 ppm in iodide **3.20** (Figure 3.6). Base induced cyclisation of **3.20**, however, again only gave starting material.

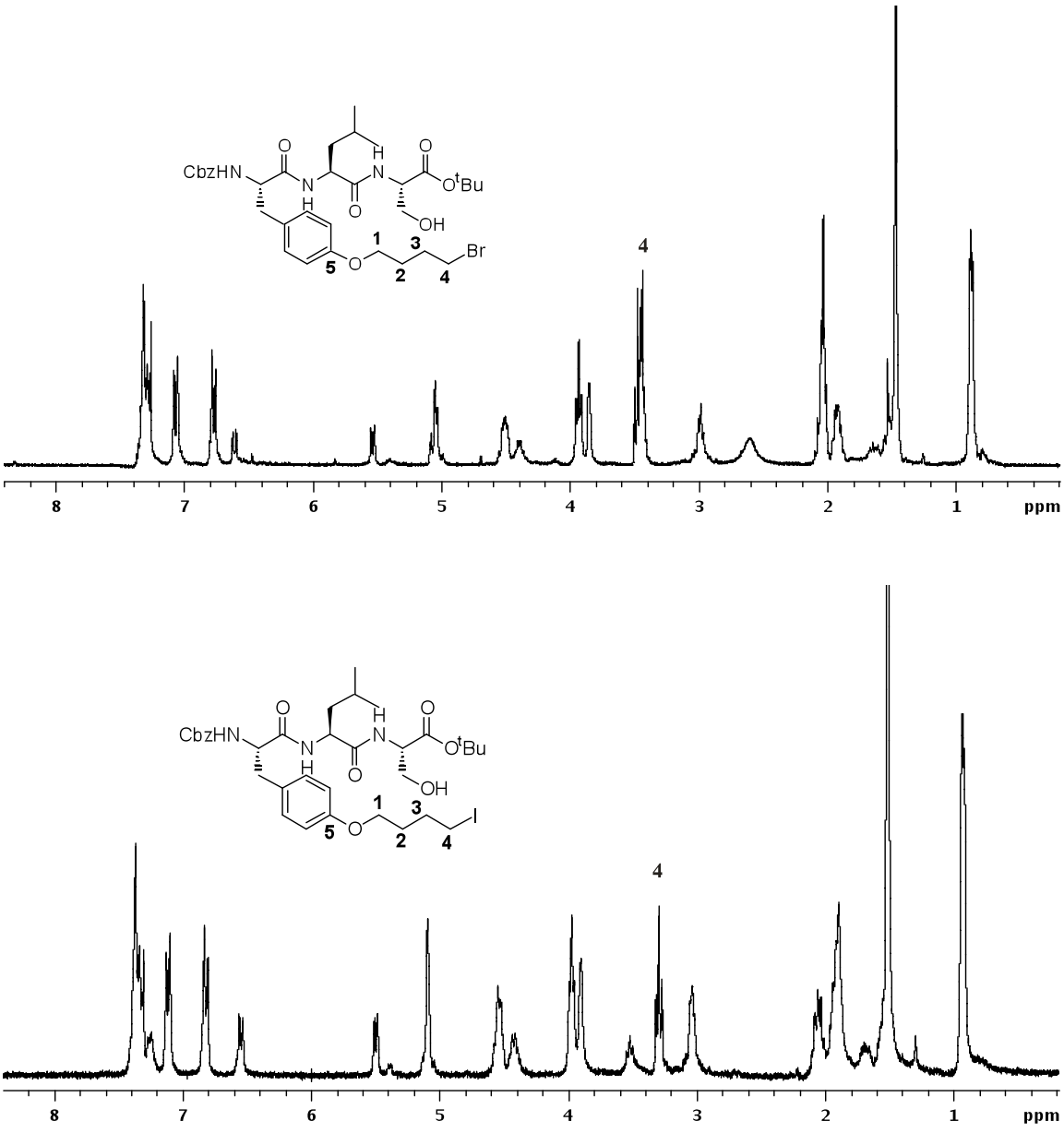


Figure 3.6. Conversion of bromide **3.18** into iodide **3.20**.

3.4: Conclusion

A new route to **CAT811** is reported in this chapter involving an intramolecular nucleophilic substitution reaction as a key step. The overall sequence required seven steps compared to nine steps in the original route involving ring closing metathesis, see Chapter 2. The RCM-based route to **CAT811** gives an overall yield of 12% which was increased to 21% using the intramolecular nucleophilic substitution sequence. In addition, this new route is economically viable as it avoids the use of Grubbs catalyst. The reaction conditions, particularly for iodination, macrocyclisation, and reduction steps, were examined and optimized to give high yields. The reaction intermediates generated were either used without purification, or were readily purified by recrystallisation to negate the need for chromatography. The new route also avoids reactions that require the use of potentially hazard and toxic reagents.

An intramolecular nucleophilic substitution methodology developed for the efficient synthesis of **CAT811**, was also applied in an attempted synthesis of 19-membered macrocycle **3.21**. This involved reaction of 1,4-dibromobutane with the phenol of tyrosine at P3 and hydroxyl group of a serine at P1 in tripeptide **3.16**. Alkylation of the phenol group, under the conditions developed for the synthesis of **CAT811** proceeded well in the presence of K_2CO_3 and Cs_2CO_3 to give alkyl bromide **3.18** in 67% yield. Alkylation of serine hydroxyl group with alkyl bromide was first attempted in the presence of NaH in acetonitrile; however reaction gave only starting material. Alkyl bromide **3.18** was then converted into alkyl iodide **3.20** with a better leaving group in attempt to facilitate the subsequent intramolecular cylisation, but again there was no evidence of the desired macrocycle by NMR studies of the crude reaction material.

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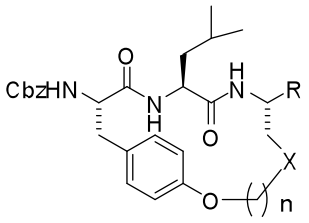
Chapter 4: Synthesis of histidine containing macrocyclic calpain inhibitors

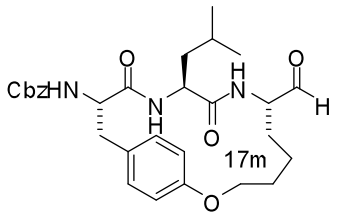
4.1: Introduction

Molecular modelling studies on the 16-19 membered macrocycles **1.20-1.23** showed that they are constrained into a *β*-strand geometry, a conformation universally adopted by inhibitors of proteases as discussed in Chapter 1.[1] These aldehydes, **1.20a-1.23a**, and the corresponding alcohols, **1.20b-1.23b**, were prepared by previous members of the research group and assayed against ovine calpain 1 and 2, with the results reported in Chapter 1. These studies showed the 17-membered aldehyde **1.21a** (**CAT811**) to be particularly potent against *o*-calpain 2 with an IC_{50} value of 30nM and a seven-fold selectivity for *o*-calpain 2 over *o*-calpain 1 (220 nM). The synthetic precursor to **CAT811**, namely alcohol **1.21b**, displayed nanomolar inhibitory activity against *o*-calpain 2 (700 nM) with less potency for *o*-calpain 1 (1750 nM). As discussed earlier, **CAT811** shows promise in the treatment of cortical cataract in lambs, with a genetic predisposition to cataract development.[2]



1.20: n=1, 16m, X=CH_2
1.21: n=2, 17m, X=CH_2
1.22: n=3, 18m, X=CH_2
1.23: n=4, 19m, X=S

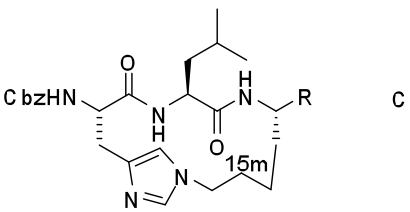
a: R=CHO
b: R=CH_2OH



CAT811

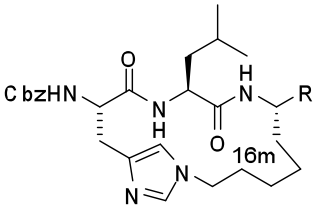
In this chapter we report a study on imidazole analogues of **CAT811**, which contain an imidazole in place of the benzene ring, in an attempt to expand the available macrocyclic protease inhibitors and to better understand the structural features that lead to potent inhibition. The imidazole ring within histidine derivatives has aromatic characteristics and an ability to be a hydrogen bond donor and acceptor.[3] Thus the incorporation of an

imidazole ring in the macrocycles **4.1-4.3** may influence binding interactions within the binding site of calpain as discussed below.

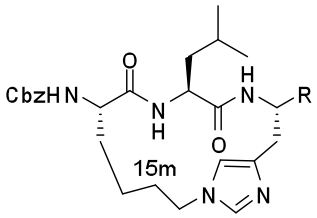


4.1

a: R=C O_2Me
b: R=C H_2OH
c: R=CHO



4.2



4.3

4.2: Molecular modelling of 4.1–4.3

A conformational search on the target macrocycles **4.1a-c**, **4.2a-c**, and **4.3a-c** was carried out at Canterbury by Wanting Jiao in order to assess their ability to adopt a *β*-strand (see Table 4.2). We were interested in using conformational analysis to determine if the position of the imidazole in the macrocycle influences the propensity of the macrocycle to adopt the critical *β*-strand geometry required for protease binding. Docking studies were also carried out using the established calpain 1 construct model developed by Dr Blair Stuart.

Table 4.2. Molecular modelling results for histidine-based macrocycles **4.1a-c**, **4.2a-c** and **4.3a-c** studies carried out with Wanting Jiao

Compounds	% *β*-strand	Essential H bonds[a]	Glide Score	Emodel Score	WHD Å
4.1a	96.10	A,C +2	-6.05	-65.5	3.51
4.1b	73.06	A,B,C +1	-7.48	-58.5	3.69
4.1c	74.57	A,B,C +1	-6.05	-64.1	3.33
4.2a	81.76	A,B +1	-4.38	-61.2	4.35
4.2b	99.11	A,B,C +3	-6.24	-60.8	3.41
4.2c	46.80	A,B,C +1	-5.72	-59.0	3.51
4.3a	12.07	A,B +1	-5.42	-60.2	5.51
4.3b	41.58	A +1	-6.17	-56.0	3.79
4.3c	4.24	NONE +3	-5.93	-50.8	10.7

[a] Hydrogen bonds from the carbonyl group of Gly_{208}, the NH group of Gly_{208}, and the carbonyl group of Gly_{271} of the o-CAPN1 homology model are labeled A, B and C respectively. [b] War head distance (WHD) is the distance between the warhead carbon and the active site cysteine sulfur in Å.

These computational studies revealed the following key points:

i. Macrocyclic alcohols **4.1b** and **4.2b** and aldehydes **4.1c** and **4.2c** exhibit three essential hydrogen bonds (denoted as A, B and C) with Gly271 and Gly208 of the active site of calpain 1 that stabilize a *β*-strand conformation of the peptide chain (Figure 4.1).

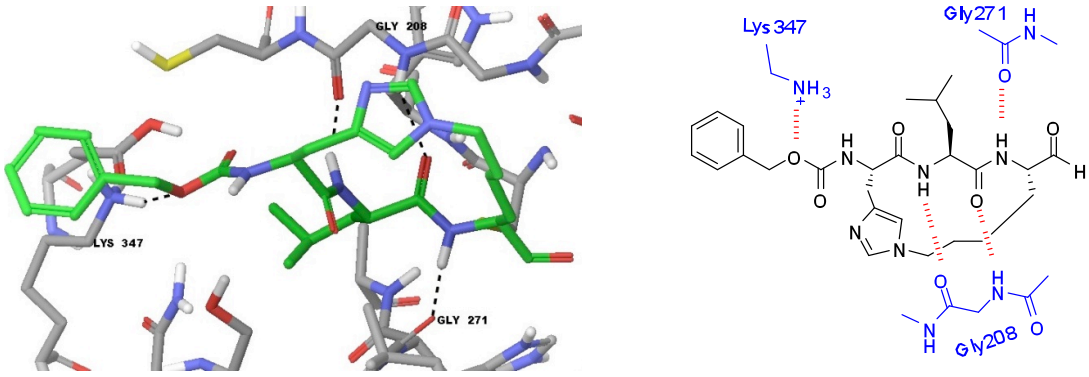


Figure 4.1. Representative bonding mode of 15-membered macrocyclic aldehyde **4.1c** with a histidine at P3.

These three hydrogen bonds are also observed in X-ray crystal structures of leupeptin in a complex with the calpain 1 construct (1TL9), **SNJ-1715** complexed with calpain 1 construct (2G8E), **E-64** conplexed with calpain 1 construct (1TL0) and **AK-295-D2** complexed with calpain 1 construct (2R9C) supporting the importance of these hydrogen bonds (see Figure 1.14, Chapter 1).

ii. The warhead distance (WHD) for aldehydes **4.1c** and **4.2c**, as defined by the distance between the warhead carbonyl carbon and the active cysteine sulfur in Å, is less than 4.5 Å. A distance of less than 4.5 Å is required for nucleophilic attack by the sulfur of cysteine for a reversible covalent inhibitor (Table 4.2).[4]

iii. All nine compounds **4.1a-c**, **4.2a-c** and **4.3a-c** have low energy Glide scores and Emodel Scores[5,6] suggesting tight binding for the protein–ligand complex (Table 4.2).

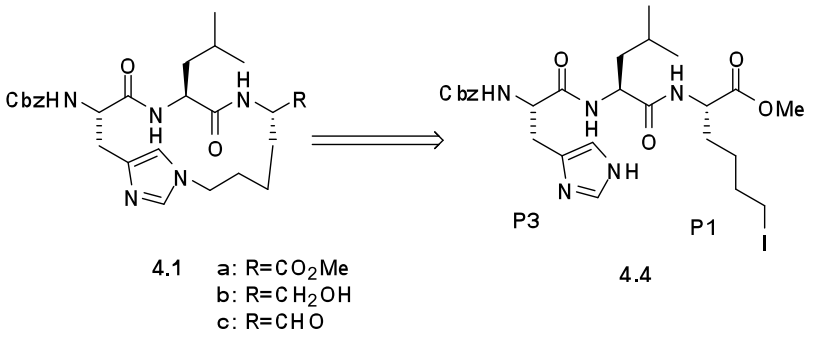
iv. The macrocycles **4.1a-c** and **4.2a**,**b** have a high percentage of *β*-strand conformers (>70%) while the macrocycles **4.2c** and **4.3b** exhibit reduced 47% and 41% *β*-strand conformers, respectively. Macrocycles **4.3a**,**c** did not adopt a *β*-strand conformation. The Boltzmann weighted percentage of *β*-strand for each macrocycle was based on the ψ (Psi) and φ (Phi) angles of the P2 leucine amino acid. Ramachandran plots of ψ, φ angles for *β*-strand or *β*-sheet regions of protein X-ray crystal structures show that typical ψ angles to be between 90º and 160º and that of φ angles to be between -90º and -160º (see Figure 1.16, Chapter 1).[7]

v. Compounds **4.3a-c** did not exhibit three essential hydrogen bonds with residues Gly271 and Gly208 of the active site binding pocket of μ-calpain. They do not appear to adopt a *β*-strand conformation.

The macrocycles **4.1a-c**, **4.2a-c** and **4.3a-c** were prepared and assayed against calpains as described in following sections in attempt to develop some structure-activity relationships and to investigate the validity of the modeling results.

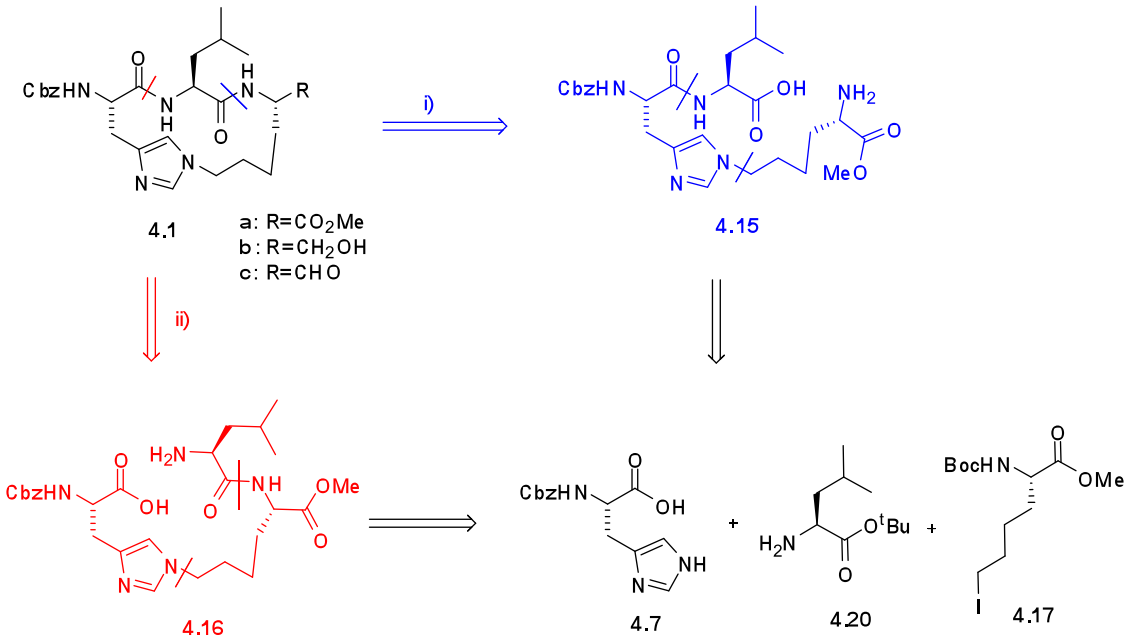
4.3: Synthesis of macrocycles 4.1-4.3

A number of routes were considered for the preparation of the macrocycles. The first involved intramolecular nucleophilic substitution as was developed for the synthesis of **CAT811**, see Chapter 3. The key macrocyclic precursor **4.4** to the 15-membered macrocycles **4.1a-c** required a histidine at P3 and a terminal alkyl iodide at P1. Macrocyclisation would then be achieved by intramolecular nucleophilic displacement of alkyl iodide with N-1 of the imidazole (Scheme 4.1). The corresponding full synthesis is shown in Scheme 4.3, however this methodology was not successful.



Scheme 4.1. Retrosynthesis of macrocycle **4.1a-c** by intramolecular nucleophilic substitution.

Two alternative routes to compounds **4.1a-c** were also investigated involving an intramolecular lactamization of either **4.15** or **4.16**, as shown in Scheme 4.2. The route (blue) involving **4.15** was investigated first and this proved successful. On this basis the alternative route involving **4.16** was not investigated.



Scheme 4.2. Retrosynthesis of macrocycles **4.1a-c** by intramolecular lactamization.

It was envisioned that the key precursor **4.15** (Scheme 4.2) would be prepared by alkylation at N-1 of the imidazole ring of Cbz-histidine **4.7** with alkyl halide **4.17** followed by coupling with leucine derivative **4.20**. Alternatively it could be prepared by coupling Cbz-histidine **4.7** with leucine **4.20** to give a dipeptide that would be alkylated on the imidazole with alkyl halide **4.17**. The intramolecular lactamization methodology developed for the synthesis of **4.1a-c** was then used to prepare **4.2a**,**b** and **4.3a-c**. This will be discussed in Sections 4.3.2 and 4.3.3.

All the proposed syntheses of N-1 substituted histidine containing macrocycles **4.1**, **4.2** and **4.3** required specific alkylation at the N-1 of an imidazole of histidine derivatives as shown in Figure 4.2. However, imidazoles exist as a tautomeric mixture and as such alkylation can occur at either N-1 or N-3. The difficulty in separating such a mixture renders this route unattractive.

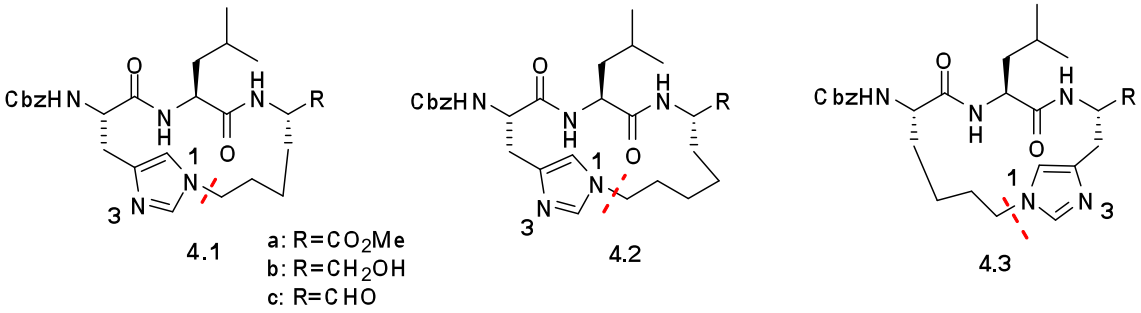


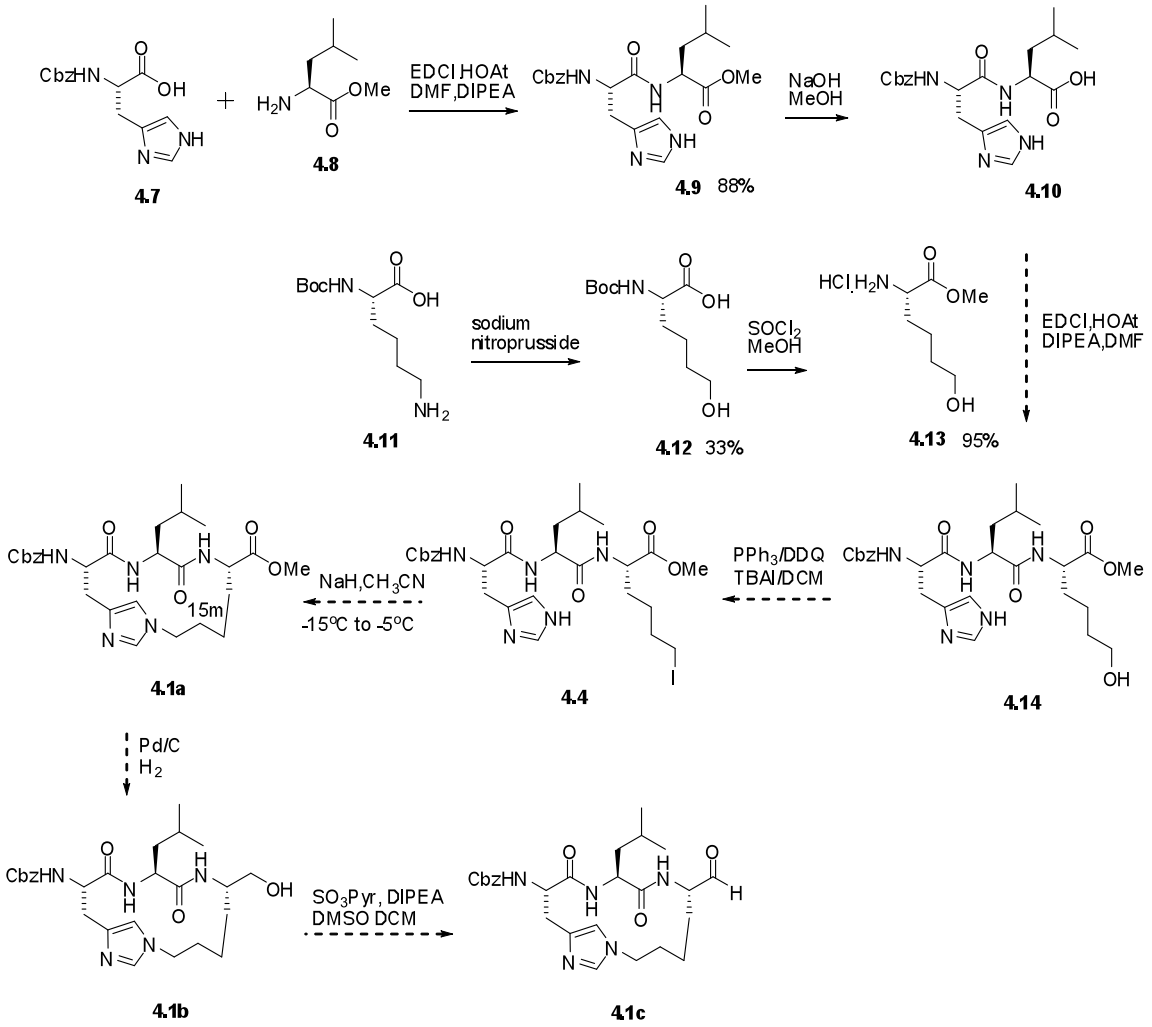
Figure 4.2. N(1)-alkyl-histidine containing macrocycles **4.1**, **4.2** and **4.3**.

However, it has been reported that alkylation of the imidazole of *N*-Boc-His with alkyl halide (RX) occurs specifically at N-1 using the base sodium hydride at -15^{o}C in DMF.[8] This suggests that it should be possible to prepare our target macrocycles under similar conditions.

4.3.1: Synthesis of 15-membered macrocycles 4.1a-c

The synthesis of the 15-membered macrocycles **4.1a-c** by intramolecular nucleophilic substitution was first attempted as shown in Scheme 4.3. The sequence began as follows. EDCI-mediated coupling of commercially available Cbz-histidine **4.7** with Leu-OMe **4.8** gave dipeptidyl ester **4.9** in 88% yield. The methyl ester of **4.9** was hydrolysed under basic conditions, however the resulting carboxylic acid **4.10** proved to be highly water soluble and we were thus unable to extract it from the crude reaction mixture. The crude reaction product, after evaporation of the water, was therefore used in subsequent coupling with **4.13** in an attempt to prepare the less polar tripeptide ester **4.14**. The required alcohol **4.13** was prepared by the reaction of *N*-Boc-Lys-OH **4.11** with sodium nitroprusside to give **4.12** in 33% yield. This was followed by removal of *N*Boc group and esterification by treatment with thionyl chloride, in the presence of methanol, to give the hydrochloride salt **4.13**.

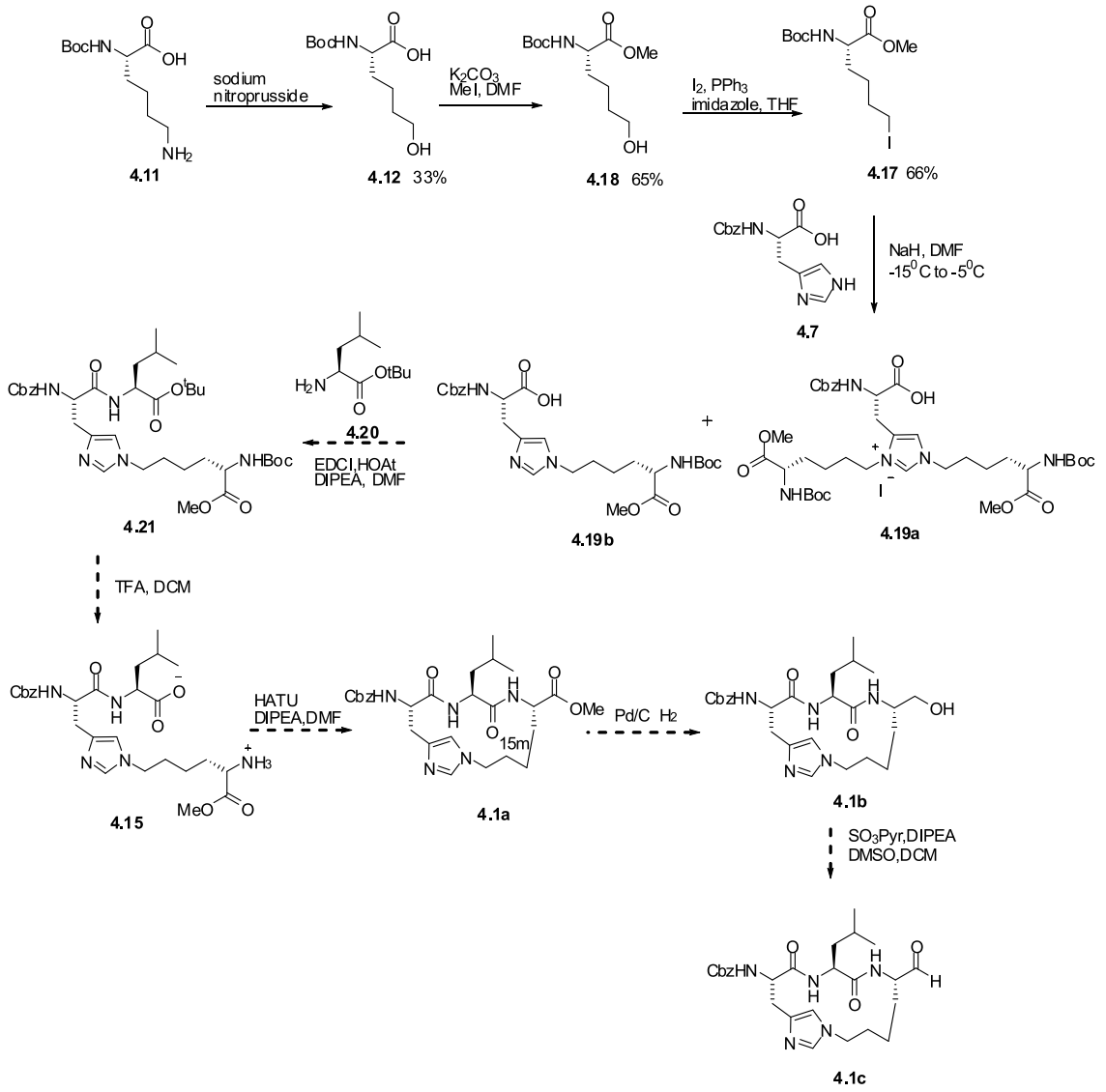
Coupling of the crude sample of dipeptide **4.10** with the hydrochloride salt **4.13**, in the presence of EDCI/HOAt, proved to be unsuccessful with only starting material being isolated. This route was not explored further because of the high aqueous solubility of **4.10** and the associated problems.



Scheme 4.3. Attempted synthesis of 15-membered histidine containing macrocycles **4.1a-c** by intramolecular nucleophilic substitution.

The next method investigated involved the intramolecular lactamization shown in Scheme 4.4. The route began with treatment of Boc-lysine **4.11** with sodium nitroprusside. Esterification of the resulting amino acid derivative **4.12**, under basic conditions, gave (*S*)-2-*tert*-butoxycarbonylamino-6-hydroxy-hexanoic acid methyl ester **4.18** in 65% yield. Iodination of **4.18** was then attempted by reaction with PPh_3, DDQ, and TBAI, not shown in the Scheme. Purification of the resulting reaction mixture by column chromatography gave only a trace amount of product **4.17** based on an NMR

analysis. The presence of **4.17** was confirmed by the signal in mass spectrum at 387.2365 m/z.



Scheme 4.4. Intramolecular lactamization for the synthesis of 15-membered macrocycles **4.1a-c**.

By contrast, iodination of **4.18** with PPh_3 (1.5 equiv), I_2 (1.5 equiv) and imidazole (1.6 equiv) gave a 66% yield of **4.17** after purification by chromatography on silica gel, see Scheme 4.4.

Alkylation of Cbz protected histidine **4.7** with alkyl halide **4.17** was then carried out in the expectation that it would occur at N-1. However, a mixture of mono- and di-alkylation was observed as revealed by mass spectrometry, see Figure 4.3. This mixture proved challenging to separate because of the high polarity of **4.19a** and **4.19b** ($C_{38}H_{58}N_5O_{12}$+ ($M^{+\cdot}$-I^-) 776.4094 m/z; calculated 776.4076 m/z) and as such this route was abandoned.

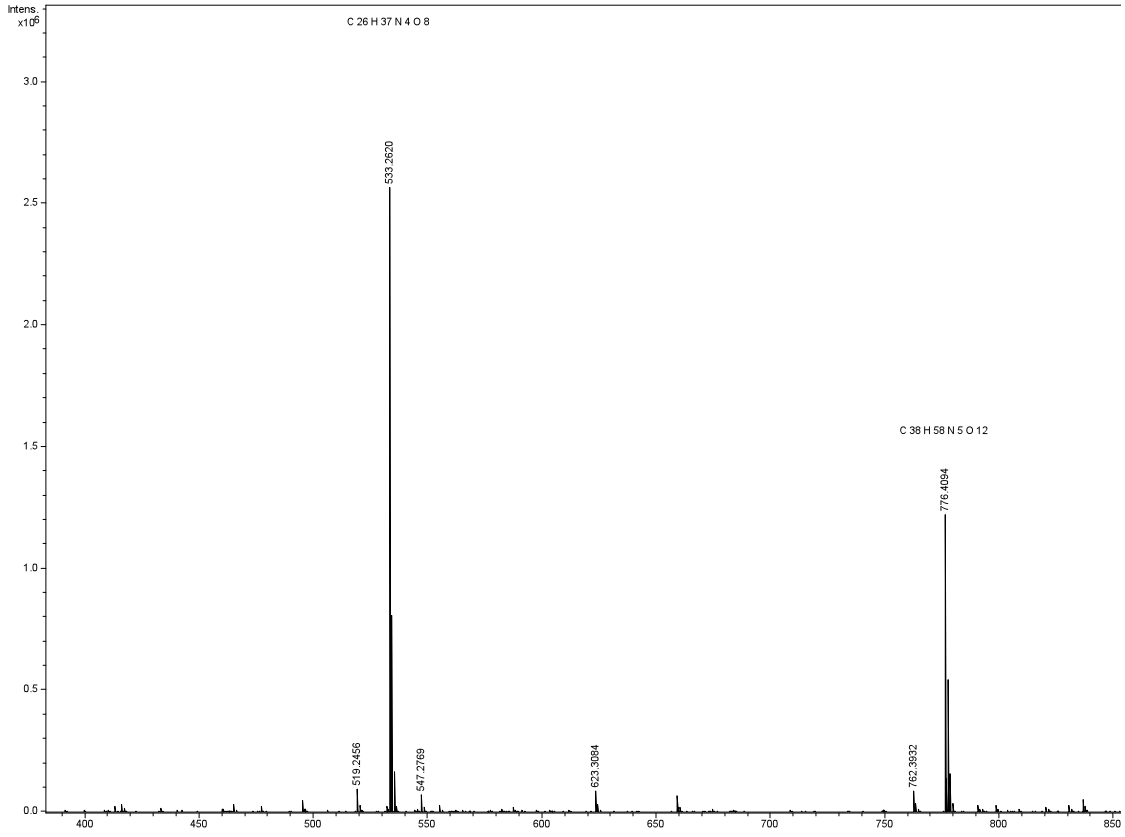
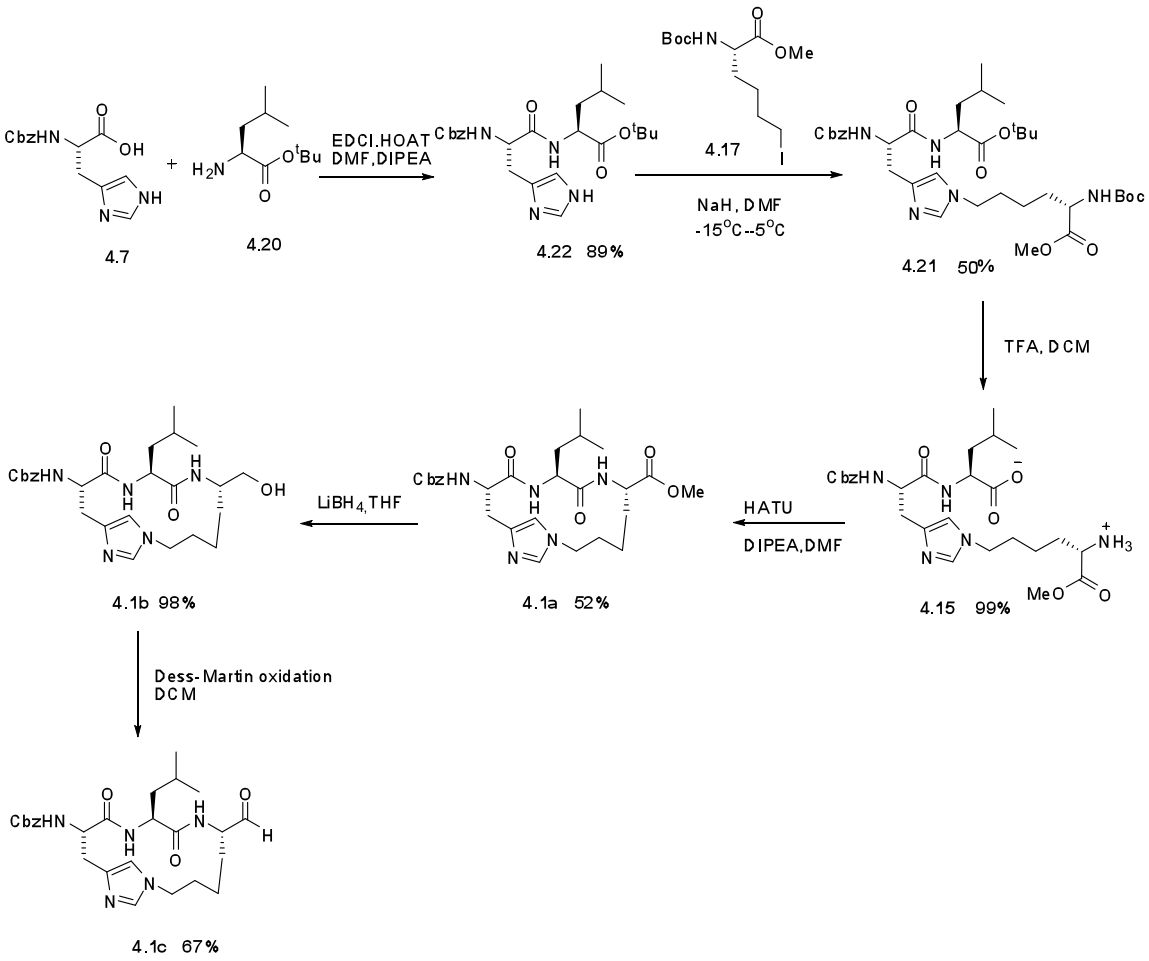


Figure 4.3. Mass spectrum of a mixture of mono and di alkylation.

A second lactamization-based route to the 15-membered macrocycles **4.1a-c** as shown in Scheme 4.5 proved to be more successful. This involved coupling of Cbz-histidine **4.7** with leucine *tert*-butyl ester **4.20** to give the dipeptide **4.22** in 89% yield. The imidazole ring of **4.22** was then deprotonated with NaH at a low temperature in DMF and the resulting anion alkylated with previously prepared alkyl halide **4.17** to give **4.21** in 50% yield after purification by chromatography on silica gel.



Scheme 4.5. Synthesis of 15-membered macrocycles **4.1a-c** *via* intramolecular lactamization.

Selective alkylation of **4.22** at N-1 to give **4.21** was confirmed using a Heteronuclear Multiple Bond Correlation (HMBC) experiment, which correlates ^{1}H and ^{13}C resonances for atoms separated by 2-3 bonds with suppression of 1-bond correlations. The HMBC spectrum of **4.21** (Figure 4.4) shows that H6 at 3.90 ppm is coupled with C7 (a 2-bond coupling), and with C8 and two imadazole carbons namely C2 (137.1 ppm) and C5 (117.8 ppm) (all involve three bond coupling). Correlation from H6 to imidazole carbon C4 is not apparent, which is a four bond coupling. These observations are consistent with the structure of compound **4.21** that is the result of specific alkylation on N-1 position of **4.22**.

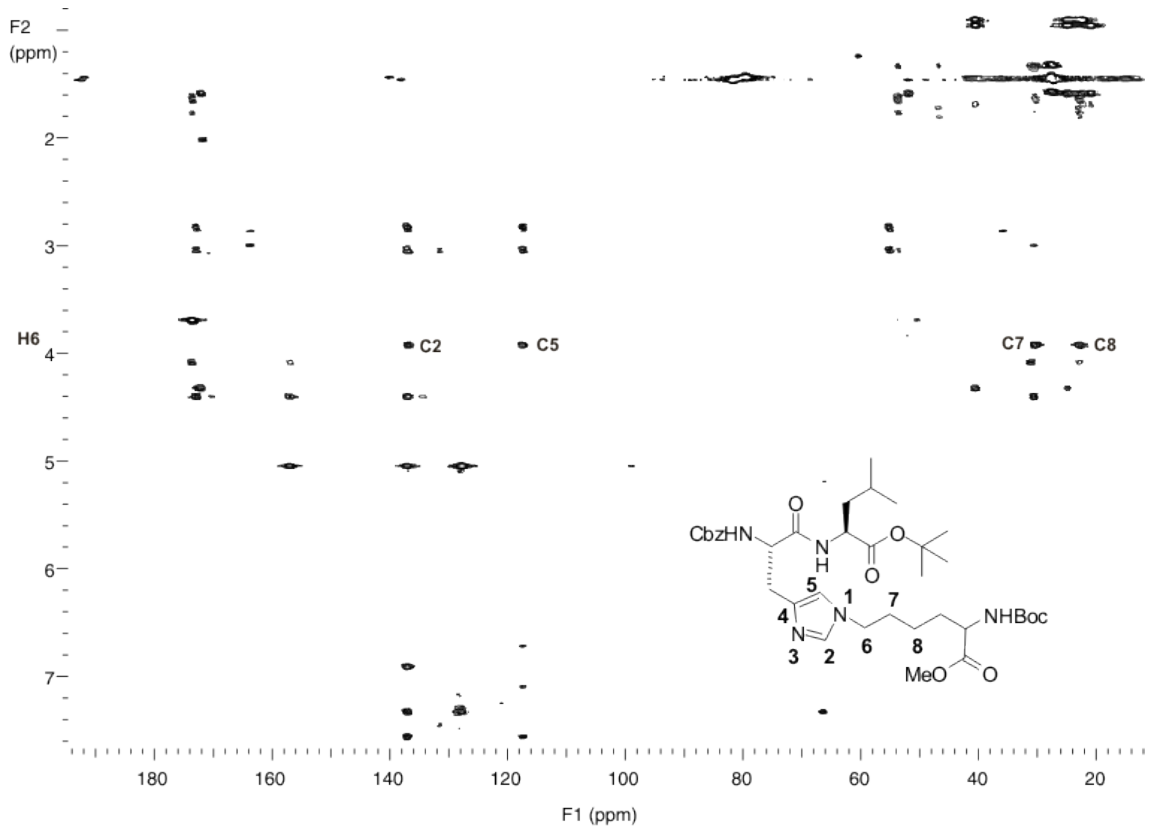
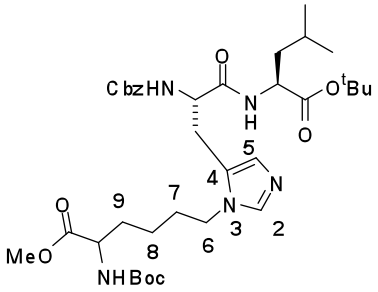


Figure 4.4. HMBC spectrum of compound **4.21**. Selected proton resonances H6 are labeled at the side of the spectrum and selected carbon resonances are labeled beside the coupled peak.

If substitution had occurred on N3 to give the alternative isomer **4.23** with its structure shown below, coupling would not be observed between H6 and the C5 of the imidazole as this represents a 4 bond distance. However, one would expect to observe coupling with C4, C2, C7 and C8. However such correlations were not observed in the HMBC spectrum (see Figure 4.4) which demonstrates that alkylation has occurred at (N-1) position of imidazole ring.



4.23

The *tert*-butyl and *N*-Boc protecting groups of **4.21** were removed by acidolysis with trifluoroacetic acid to give **4.15** in almost quantitative yield. Intramolecular lactamization of compound **4.15** was then carried out by treatment with HATU to give macrocycle **4.1a** in 52% yield after purification by chromatography. The molecular formula of tripeptide **4.1a** was confirmed by a parent ion in the mass spectrum at 528.2830 m/z. The ^{1}H NMR spectrum (Figure 4.5) shows key resonances centered at δ 4.64, 4.54, and 4.35 ppm, corresponding to the three α-protons of leucine, tyrosine and glycine derivatives in **4.1a**, respectively. Two multiplets centered at 4.23 and 4.12 ppm correspond to the H4 methylene.

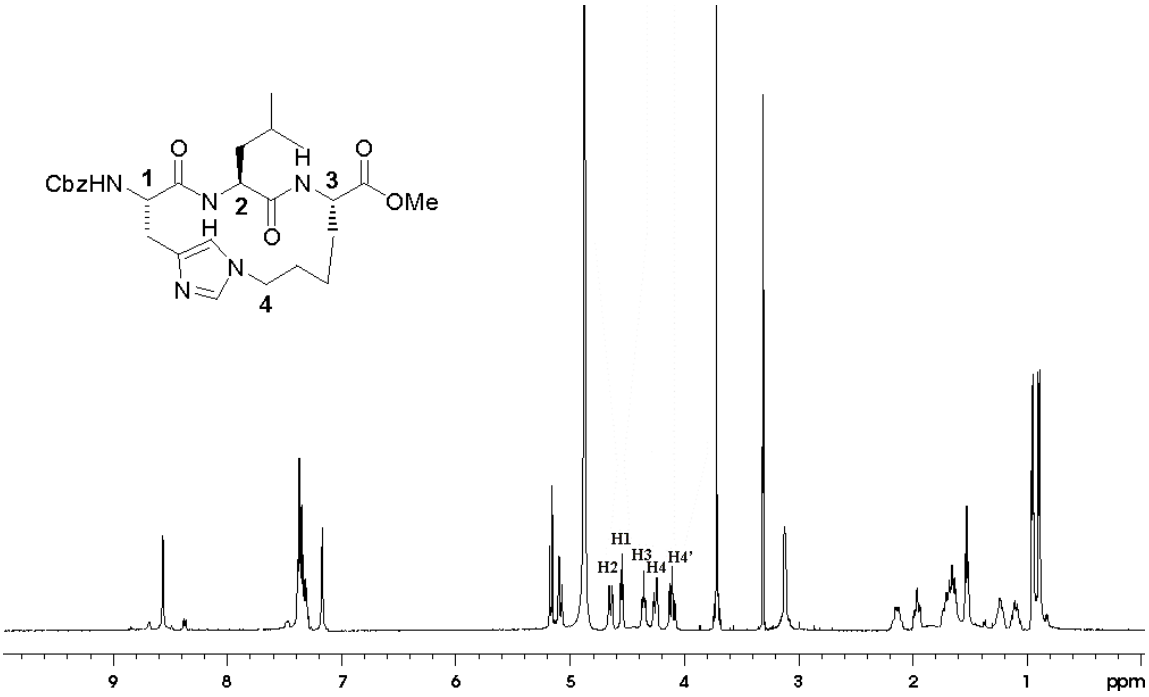
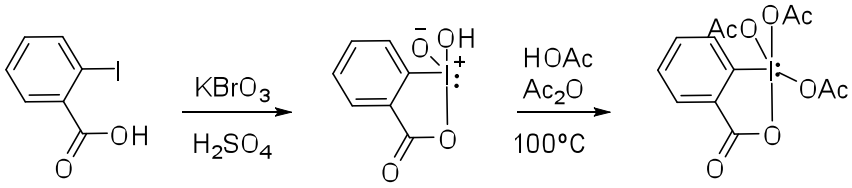


Figure 4.5. ^{1}H NMR spectrum of 15-membered macrocyclic ester **4.1a**.

The final steps in synthesis of **4.1c** involved reduction of the ester **4.1a** to the alcohol **4.1b**, and oxidation to aldehyde (see Scheme 4.5). The best established conditions developed for the reduction of **3.11** (see Table 3.2, Chapter 3) were used here. Thus reduction of methyl ester of **4.1a**, in the presence of $LiBH_4$ (2 equiv) in THF, gave **4.1b** in 98% yield after recrystallisation from ethyl acetate. The structure of the alcohol **4.1b** was confirmed by ^1H NMR spectroscopy, with the absence of a resonance for a methyl ester (as in **4.1a**) and the appearance of resonances centered at 3.16 and 3.23 ppm corresponding to two protons adjacent to the hydroxyl group.

Oxidation of primary alcohol **4.1b** was first attempted using DMSO and sulphur trioxide-pyridine complex as the oxidant. However, this gave only starting material. DMSO-based oxidation on a small scale has been reported[9] using a sacrificial amount of iso-propyl alcohol to allow larger quantities of reagent to be used. However, attempted oxidation under these conditions gave only returned starting material.

Dess-Martin periodinane **4.26** has been reported[10] for the oxidation of primary and secondary alcohols to the corresponding aldehyde or ketone. The preparation of this reagent is shown in Scheme 4.6. 2-Iodobenzoic acid **4.24** was treated with potassium bromate in sulphuric acid to give the cyclic tautomer of 2-iodobenzoic acid **4.25** (93%). Treatment of the intermediate **4.25** with acetic anhydride and acetic acid gave the desired Dess-Martin periodinane **4.26** in 72%.



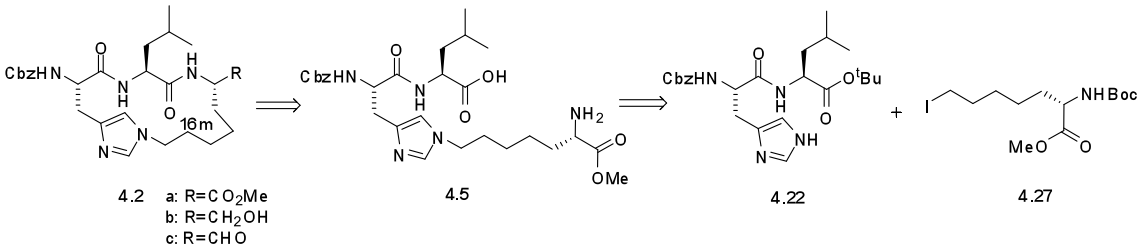
4.24 **4.25** 93% **4.26** 72%

Scheme 4.6. Synthesis of Dess-Martin periodinane **4.26**.

Dess-Martin oxidation of **4.1b**, in the presence of **4.26** (2 equiv) in dichloromethane, was successful and gave the desired aldehyde **4.1c** however in a low yield (< 10%) using Dess-Martin periodinane that I have prepared. A sample was send to Ashok Pehere in Adelaide who used a commercially available Dess-Martin periodinane **4.26** and obtained a improved yield (67%). .

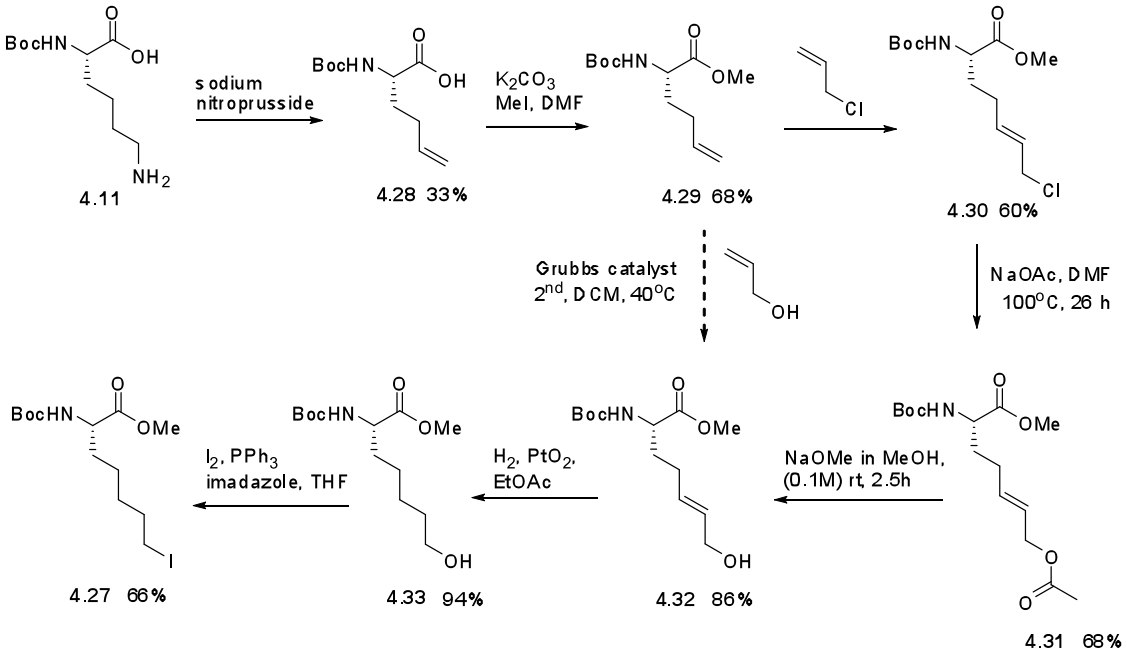
4.3.2: Synthesis of 16-membered macrocycles 4.2a,b

The methodology developed to prepare the 15-membered macrocycles **4.1a-c** was then applied to synthesis of 16-membered macrocycles **4.2a-c** as shown in Scheme 4.7. The key acyclic precursor **4.5** was prepared by alkylation of Cbz-His-Leu-O^{t}Bu **4.22** with alkyl halide **4.27**.



Scheme 4.7. Retrosynthesis of 16-membered histidine containing macrocycles **4.2a-c**.

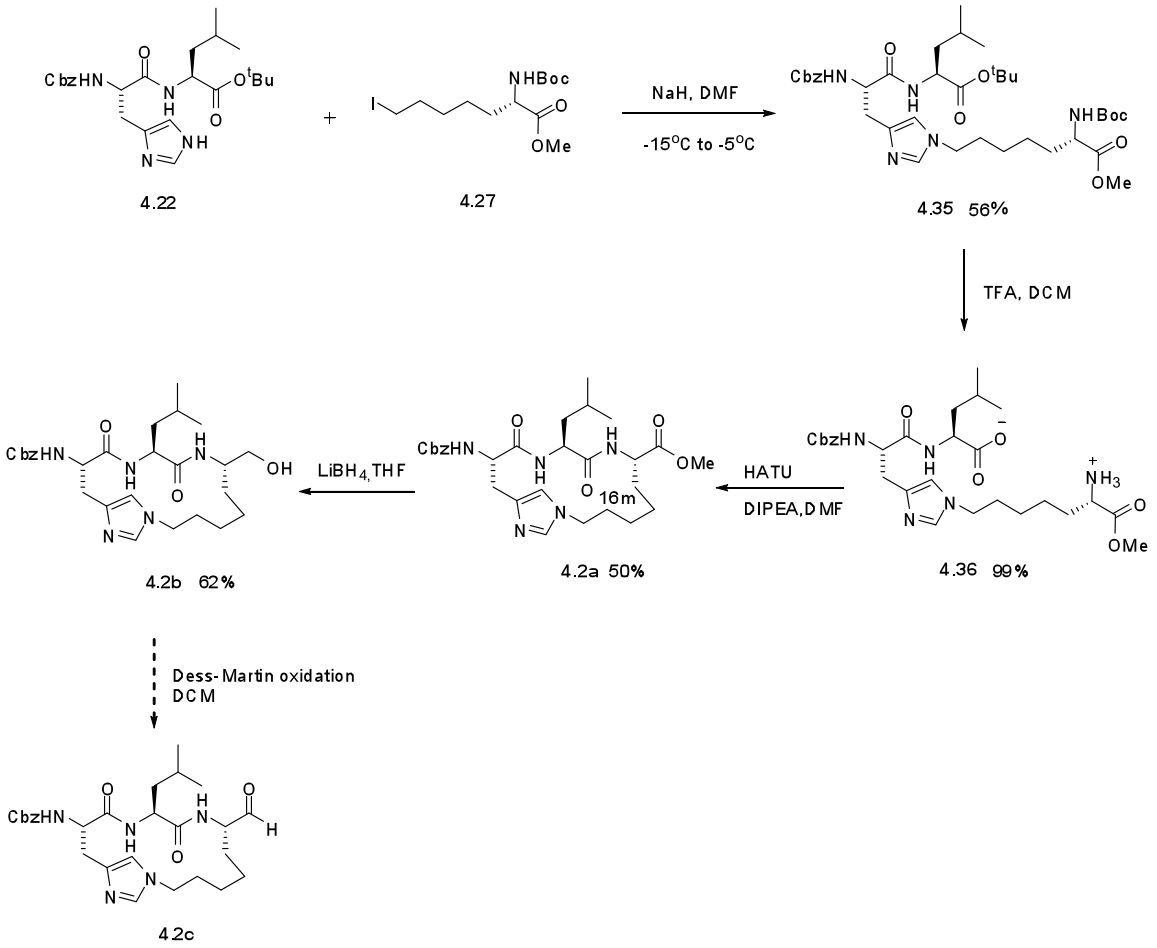
The iodo compound **4.27** was prepared from Boc-lysine **4.11** as shown in Scheme 4.8. Treatment of **4.11** with sodium nitroprusside, followed by methylation with methyl iodide gave (*S*)-2-*tert*-butoxycarbonylamino-hex-5-enoic acid methyl ester **4.29** in 68% yield. Cross metathesis of **4.29** with allyl alcohol in presence of the second generation Grubbs catalyst gave a poor yield (< 10%) of (*S*)-2-*tert*-butoxycarbonylamino-7-hydroxy-hept-5-enoic acid methyl ester **4.32**. The proposed route to **4.27** involving cross metathesis of **4.29** with allyl alcohol was abandoned because of this low yield.



Scheme 4.8. Synthesis of alkyl iodide **4.27**.

However, an alternative route to **4.27**, involving cross metathesis of **4.29** with an excess allyl chloride and 10 mol% Grubbs catalyst as a key step, proved to be successful (see Scheme 4.8). This gave (*S*)-2-*tert*-butoxycarbonylamino-7-chloro-hept-5-enoic acid methyl ester **4.30** in 60% yield. The chloride **4.30** was converted to alcohol **4.32** in two steps according to the Eustache's method.[11]. Hydrogenation of the allylic alcohol **4.32**, over platinum oxide in a hydrogen atmosphere, gave the saturated alcohol **4.33** in 92% yield.[12] Iodination of the primary alcohol of **4.33** in presence of iodine, PPh_3 and imidazole gave **4.27** in 61%.

The imidazole N-1 of dipeptide **4.22** was deprotonated with sodium hydride at -15°C in DMF and the resulting anion alkylated with alkyl halide **4.27** (2 equiv) to give **4.35** in 56% yield (see Scheme 4.9). The molecular formula of this was confirmed by a parent ion in a mass spectrum at 716.4 m/z.



Scheme 4.9. Synthesis of 16-membered macrocycles **4.2a** and **4.2b** with intramolecular lactamization.

Monoalkylation occurred at N-1 of **4.22** to give **4.35** as confirmed by a HMBC experiment. The HMBC spectrum of **4.35** (Figure 4.6) reveals correlations from H6 (3.98 ppm) to C7 (30.3 ppm) (2-bond coupling), and also to C8 (25.7 ppm) C2 (136.1 ppm) and C5 (118.1 ppm) which are three bond coupling. H6 is not coupled with the quaternary center C4 (137.0 ppm) which is four bonds removed.

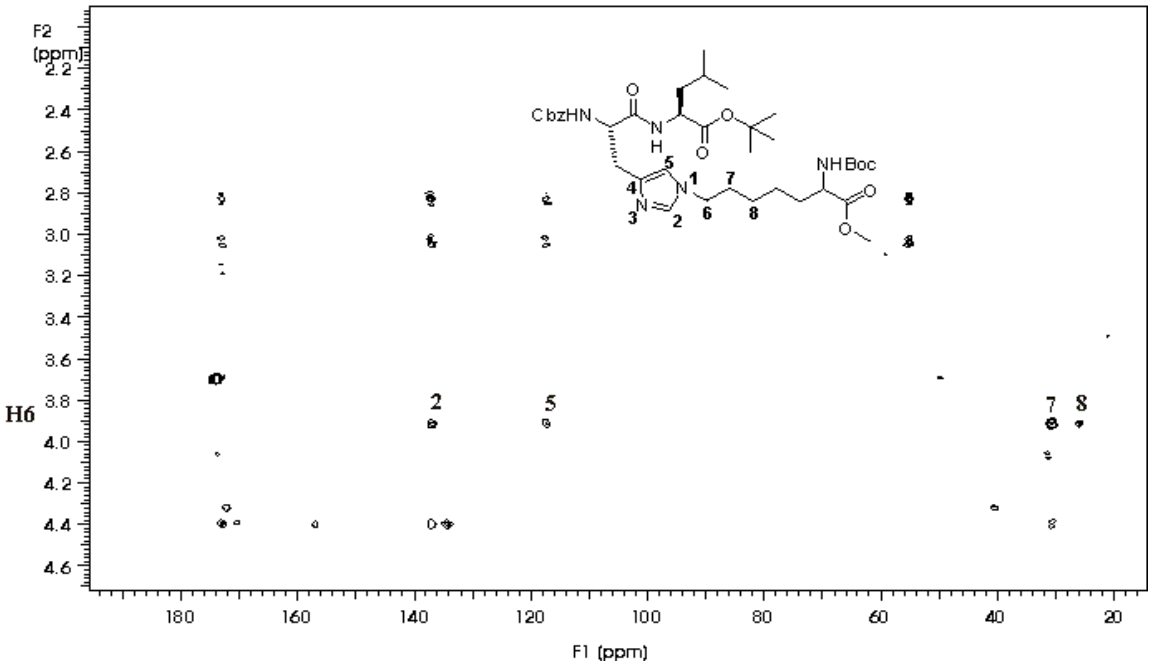
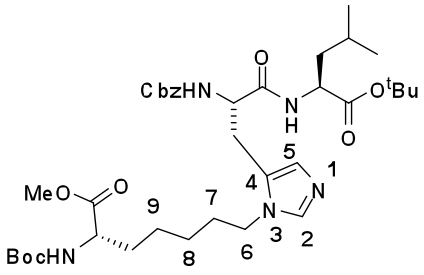


Figure 4.6. HMBC spectrum of compound **4.35**. Selected proton resonances H6 are labelled at the side of the spectrum and selected carbon resonances are labelled beside the coupled peak.

If alkylation had occurred on N-3 of **4.22** to form the isomer **4.37**. H6 would also have been coupled with imidazole carbon C4 (3-bond coupling) but not with C5, a 4-bond coupling. However these correlations were not observed in the HMBC spectrum (Figure 4.6) that demonstrates the alkylation did not occur on N-3.



4.37

The *tert*-butyl and Boc protecting groups of **4.35** were removed by acidolysis, in the presence of trifluoroacetic acid, to give **4.36** in nearly quantitative yield. Intramolecular lactamization of **4.36**, in the presence of HATU, then gave the macrocycle **4.2a** in 50% after purification by chromatography. The formation of **4.2** was confirmed by the presence of a signal in its mass spectrum at 542.2979 m/z. The ^{1}H NMR spectrum (Figure 4.7) shows the presence of three key overlapping α-protons at δ 4.42-4.52 ppm.

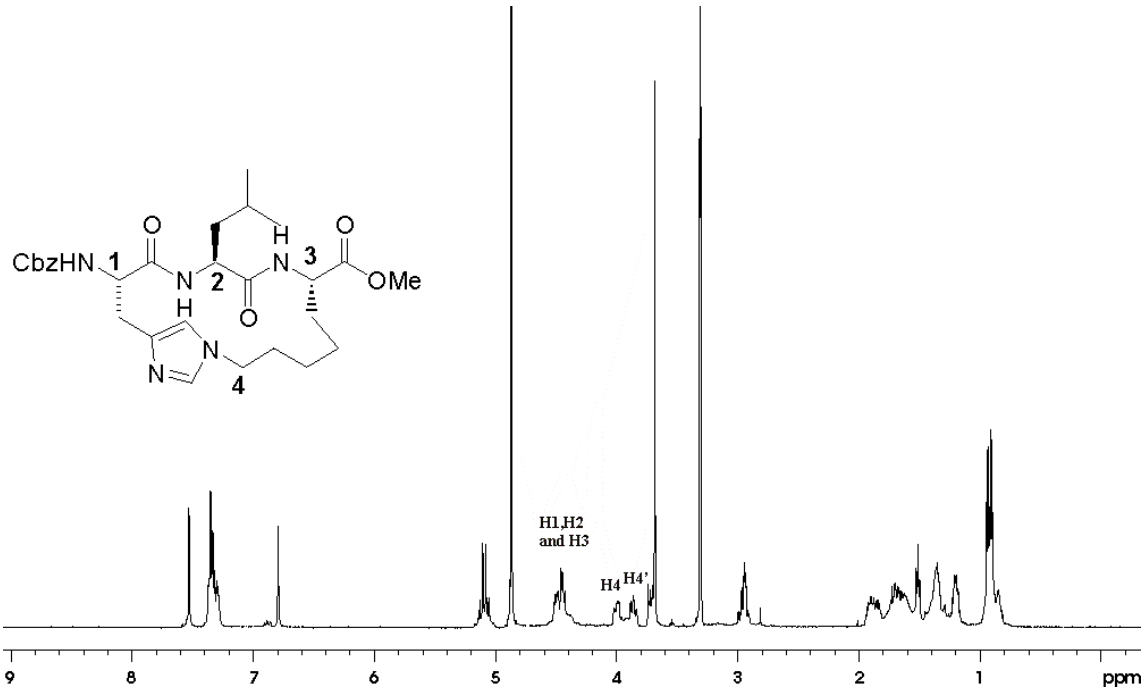
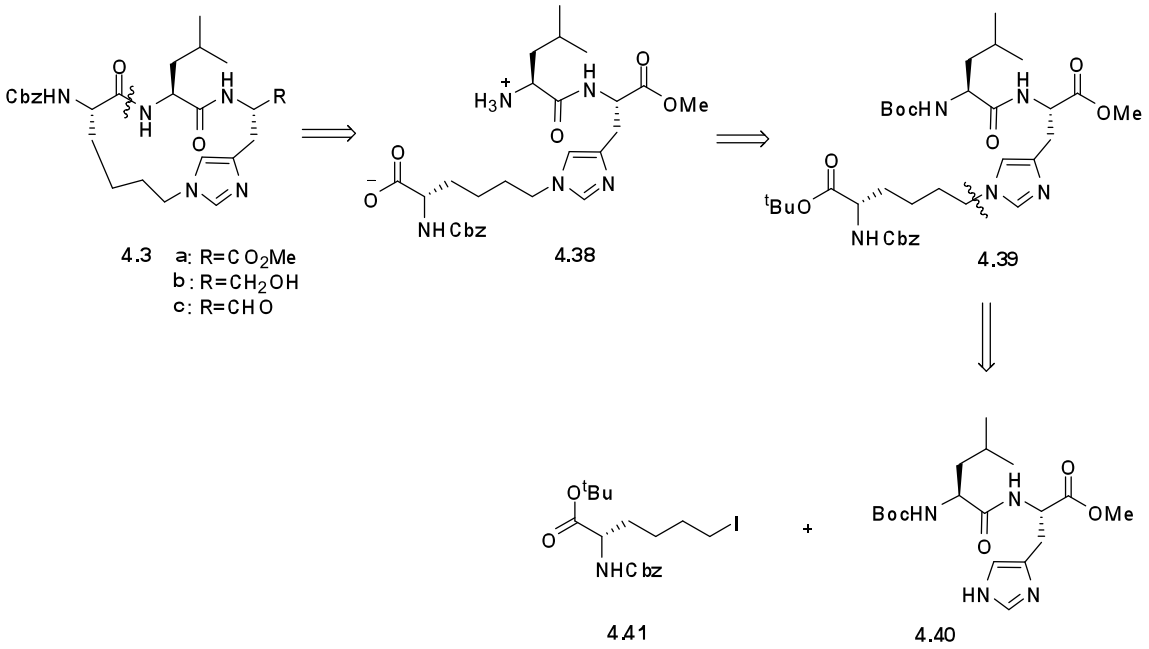


Figure 4.7. ^{1}H NMR of 16-membered macrocycle **4.2a**.

Reduction of the methyl ester of **4.2a** with $LiBH_4$ (2 equiv) in THF resulted in a 62% conversion to the alcohol **4.2b** as determined by ^{1}H NMR analysis of the crude product. However purification by column chromatography and recrystallisation failed to give the pure compound. Oxidation of crude alcohol **4.2b** to **4.2c** was therefore not attempted.

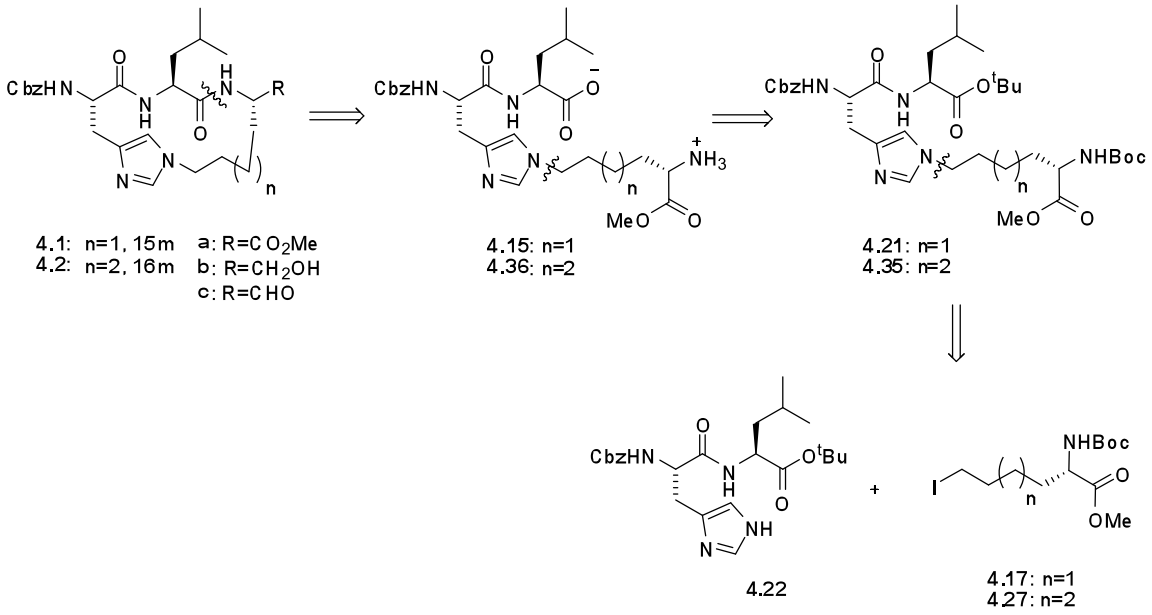
4.3.3: Synthesis of 15-membered macrocycles 4.3a-c

The intramolecular lactamization was applied to the synthesis of macrocycle **4.3a-c**. A retrosynthetic analysis of **4.3a-c** (Scheme 4.10) suggests the pseudotripeptide **4.39** as a key intermediate, with the amine and carboxyl groups protected by *N*-Boc and *t*-butyl group, respectively. Both protecting groups are acid sensitive and can thus be simultaneously removed on treatment with trifluoroacetic acid to give **4.38**. Intramolecular lactamization would then give the macrocycles **4.3a-c**. The intermediate **4.39** would be prepared by alkylation of the imidazole N-1 of **4.40** with alkyl iodide **4.41**.



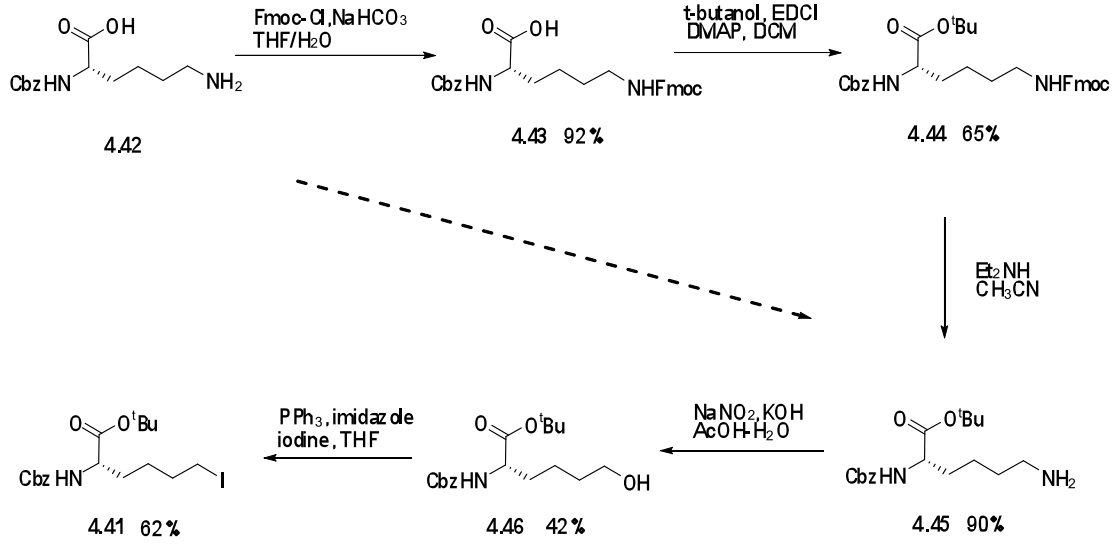
Scheme 4.10. Retrosynthetic analysis of macrocycles **4.3a-c** with histidine at P1.

Such a protection methodology was also used in the synthesis of macrocycles **4.1a-c** and **4.2a**,**b** (Scheme 4.11) as previously discussed in sections 4.3.1 and 4.3.2.



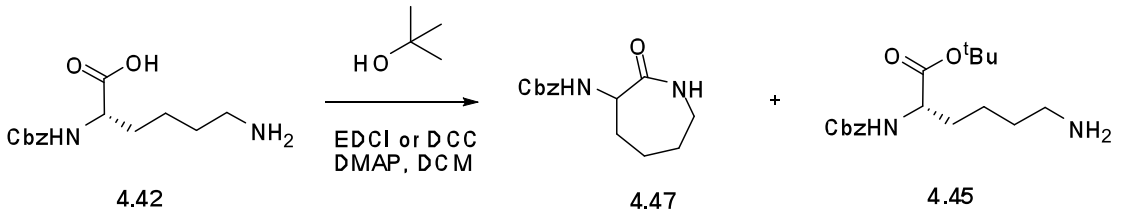
Scheme 4.11. Retrosynthesis of macrocycles **4.1a-c** and **4.2a-c** with histidine at P3.

The alkyl iodide **4.41** was prepared from the commercially available *N*-Cbz-Lys-OH **4.42** as shown in Scheme 4.12. The synthetic route required esterification of carboxylic acid of **4.42** with *t*-butyl alcohol to give **4.45**, the primary amine of which was converted to iodide **4.41**.



Scheme 4.12. Synthesis of alkyl iodide **4.41**.

However esterification of **4.42** to **4.45**, in the presence of EDCI or DCC, may lead to an intramolecular lactamization to give a seven-membered byproduct **4.47**, see Scheme 4.13. Therefore the free amine of **4.42** was first protected with a 9-fluorenylmethyloxycarbonyl (FMOC) protecting group that is stable towards acid and catalytic hydrogenation, but readily removed under mildly basic conditions.[13] Reaction of the primary amine of **4.42** with 9-fluorenylmethyl chloroformate (Fmoc-Cl) gave **4.43** in 92% yield (Scheme 4.12).



4.42 4.47 4.45

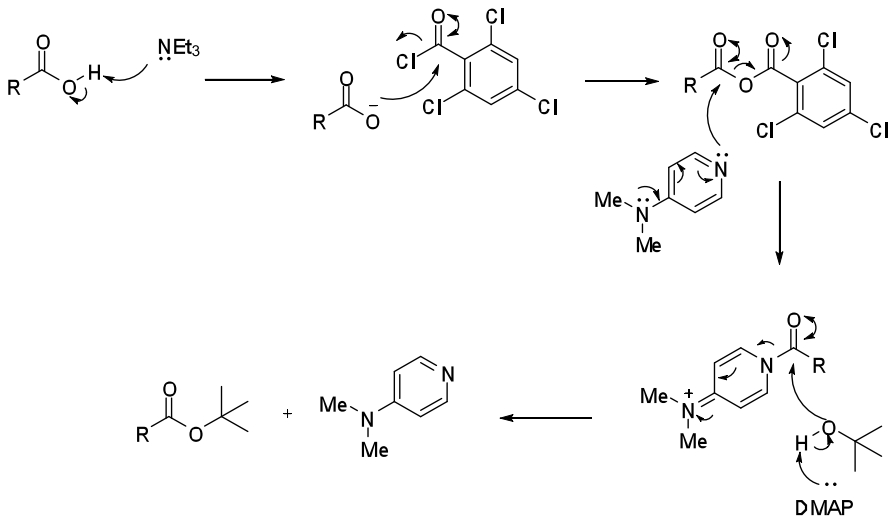
Scheme 4.13. A side reaction derived from the EDCI-mediated esterification of **4.42**.

Esterification of **4.43** with *t*-butyl alcohol was investigated using three coupling reagents as shown in Table 4.3.

Table 4.3. Conditions for *tert*-butyl esterification of carboxylic acid **4.43**.

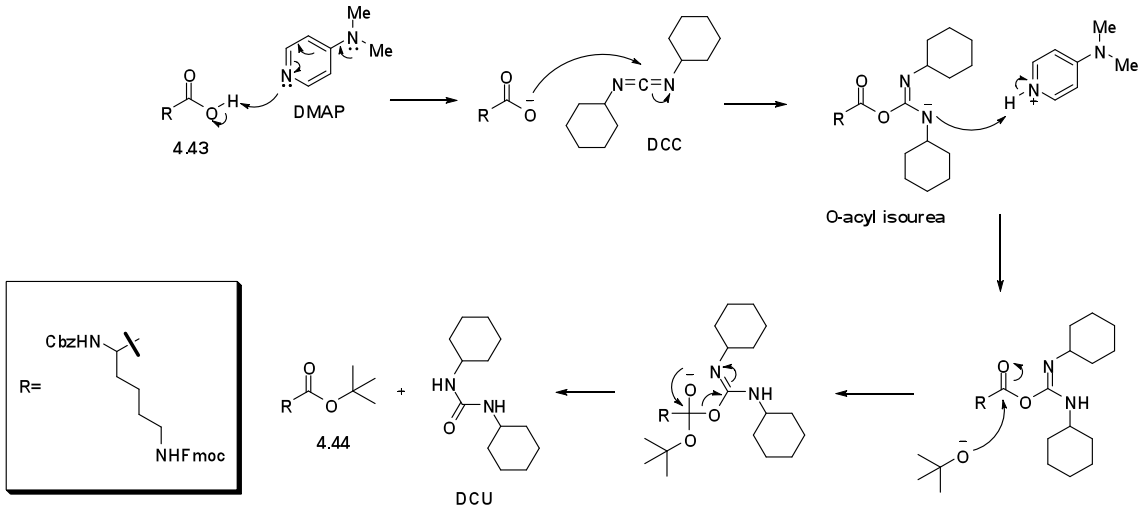
Coupling reagents	Solvents	Yields
2,4,6-trichlorobenzoyl chloride, TEA, DMAP	THF	0%
DCC, DMAP	DCM	45%
EDCI, DMAP	DCM	65%

Yamaguchi esterification[14] was attempted first. Here the carboxylic acid is reacted with 2,4,6-trichlorobenzoyl chloride in the presence of triethylamine and the resulting anhydride reacted with *t*-butyl alcohol, in the present of DMAP, to give the *t*-butyl ester. However treatment of **4.43** under these conditions (Scheme 4.14) gave starting material as the only isolatable material.



Scheme 4.14. Yamaguchi esterification.

A second method involving reaction of **4.43** with DCC/DMAP and *tert*-butanol gave the desired product **4.44** in 45% yield (see Scheme 4.15). The formation of **4.44** was confirmed by the presence of a singlet at δ 1.46 ppm in its ^{1}H NMR spectrum corresponding to the *tert*-butyl ester group. A third method, involving esterification of **4.43** with *tert*-butanol and EDCI and DMAP, proved to be the best coupling conditions and gave **4.44** in a 65% yield.[15]

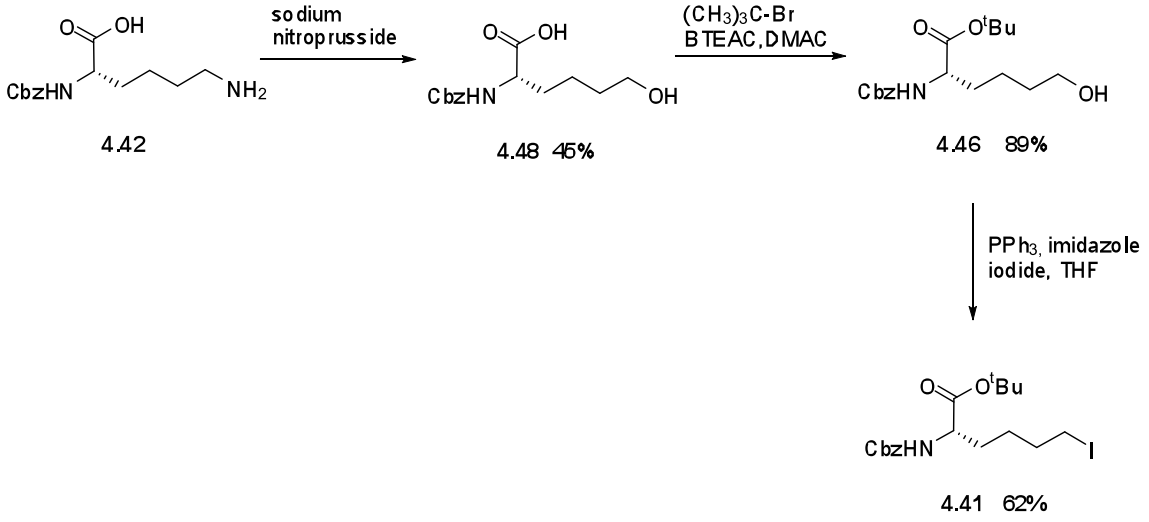


Scheme 4.15. DCC mediated *tert*-butyl esterification.

The Fmoc protecting group of **4.44** was removed on treatment with excess diethylamine in acetonitrile to give **4.45** in 90% yield, see Scheme 4.12. Diazotization of **4.45** gave hydroxy amino acid **4.46** (42%).[16] Iodination of primary alcohol of **4.46** in the presence of iodine, PPh_3 and imidazole gave alkyl iodide **4.41** in 62% yield. This route to **4.41** (Scheme 4.12) required 6 steps and resulted in an overall yield of 14%.

A more efficient preparation of **4.41** was effected by diazotization of primary amine **4.42** to form the corresponding alcohol **4.48** in 45% yield, see Scheme 4.16. Esterification of **4.48** with *tert*-butyl bromide, in dimethylacetamide (DMAC) in the presence of benzyltriethylammonium chloride (BTEAC) and potassium carbonate, gave **4.46** in 89%.[17] Iodination of **4.46**, in the presence of PPh_3, imidazole and iodine, gave the desired alkyl iodide **4.41** in 62%.

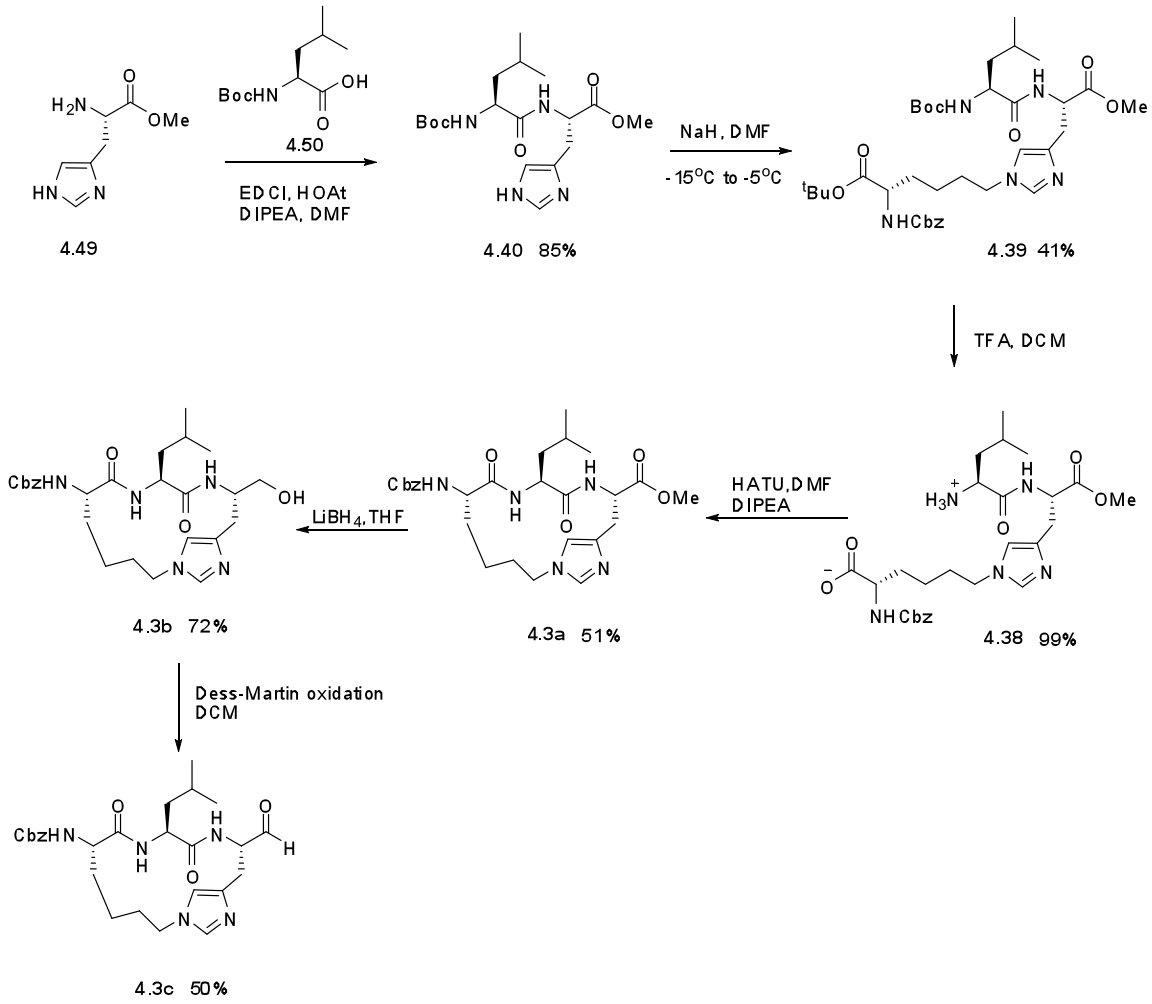
This sequence to **4.41** (Scheme 4.16) involves three steps and proceeds in an improved overall yield of 25%.



Scheme 4.16. A more efficient route to the synthesis of alkyl iodide **4.41**.

Coupling of commercially available His-OMe **4.49** with Boc-Leu-OH **4.50**, in presence of EDCI/HOAt, gave dipeptide **4.40** in 85% yield (Scheme 4.17). The alkylation of the

imidazole ring of **4.40** with alkyl halide **4.17** was carried out in the presence of NaH in DMF to give the pseduotripeptide **4.39** in 41% yield. The molecular formula of **4.39** was confirmed by a parent ion at m/z 702.4044 in the mass spectrum.



Scheme 4.17. Synthesis of 15-membered histidine containing macrocycles **4.3a-c**

The specificity of the alkylation at N-1 of dipeptide **4.40** was determined using established methodology discussed in Sections 4.3.1 and 4.3.2. An HMBC spectrum (Figure 4.8) shows that H6, at δ 3.94 ppm, is coupled with C7 (29.8 ppm) and C8 (22.6 ppm) and with the imidazole carbon C5 (117.2 ppm) and C2 (136.7 ppm). However H6 is not coupled with C4 (137.0 ppm) which is four bonds removed. These observations are

consistent with the structure of compound **4.39** that is the result of specific alkylation on N-1 position of **4.40**.

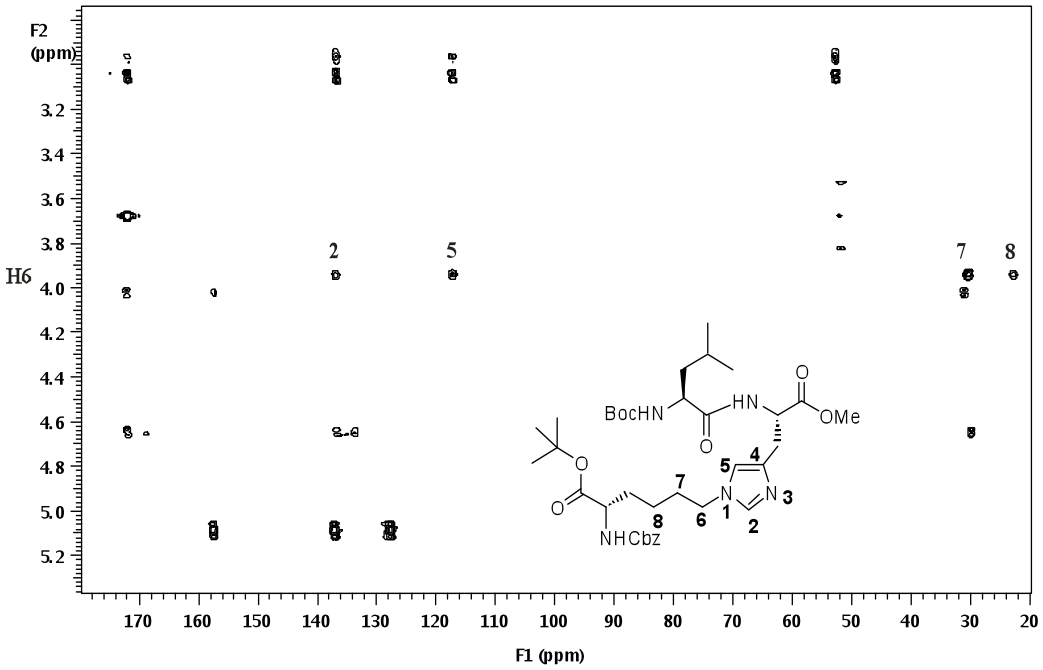
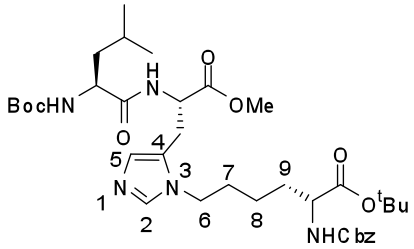


Figure 4.8. HMBC spectrum of compound **4.39**. Selected proton resonance H6 is labelled at the side of the spectrum and selected carbon resonances are labelled beside the coupled peak.

If alkylation had occurred on the N-3 position to form an alternative isomer **4.51** as shown below, H6 would be coupled with C4 of the imidazole ring which is a 3-bond coupling, but not with C5 (a 4-bond coupling). However such correlations conflict with the assigned HMBC spectrum, see Figure 4.8.



4.51

Cyclisation of **4.39** was achieved by first the removal of its Boc and *t*-butyl groups followed by HATU mediated lactamization to give the macrocyclic methyl ester **4.3a** in an overall yield of 51% (Figure 4.9). Reduction of the ester of **4.3a**, with $LiBH_4$ in THF, gave alcohol **4.3b** in 72% yield. Dess-Martin oxidation of **4.3b** was carried out by Ashok Pehere to give aldehyde **4.3c** in 50% yield.

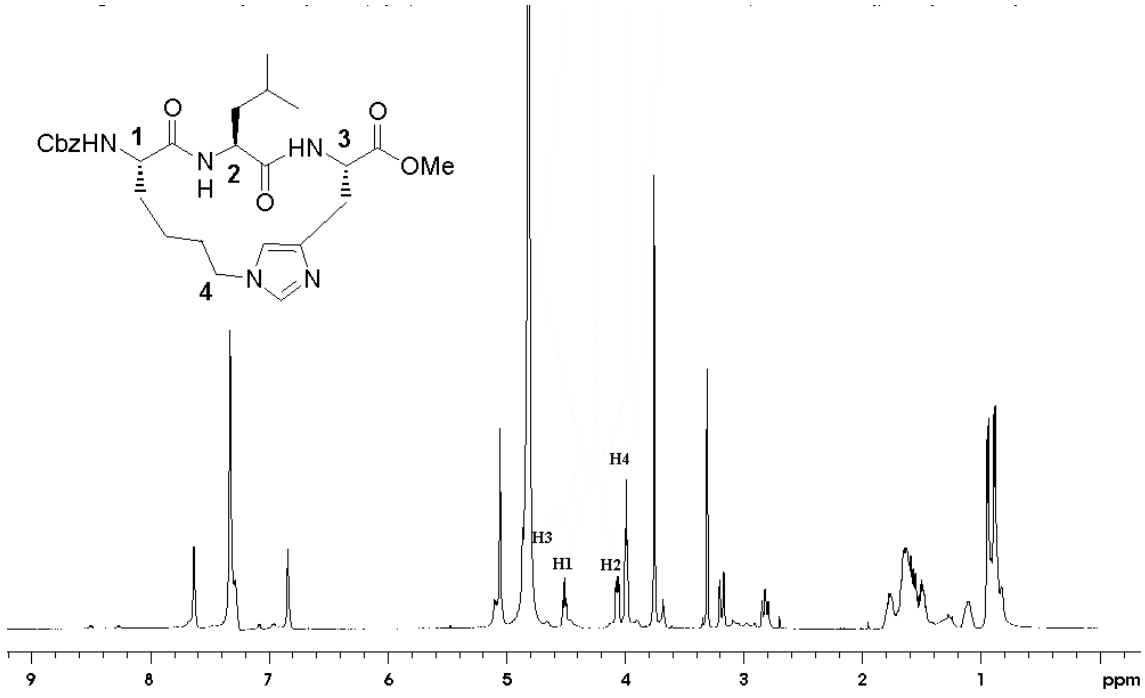


Figure 4.9. ^{1}H NMR spectrum of macrocycle **4.3a**.

4.4: Biological assay of macrocycles 4.1a-c, 4.2a,b and 4.3a-c

The Christchurch earthquake has resulted in delays in obtaining the assay results. This data has come to hand as I print the thesis. Macrocycles **4.1-4.3** were assayed against ovine calpain 2 by Dr Ondrej Zvarec. Esters **4.1a-4.3a** show no activities (IC_{50} > 50 μM).

Aldehyde **4.3c** has a moderate inhibitory activity with an IC_{50} of 1 μM and the corresponding alcohol **4.3b** shows no activity, consistent with the modelling reported above which indicated that these two compounds did not adopt a *β*-strand conformation in the docking studies.

Aldehyde **4.1c**, on the other hand, shows significant inhibitory activity with an IC_{50} of 238 nM but as expected the corresponding alcohol **4.1b** shows little activity (IC_{50} = 29 μM). Modelling studies showed that both the aldehyde **4.1c** and the alcohol **4.1b** on docking can form a *β*-strand with appropriate H-bonding interactions. The aldehyde is more active than the alcohol due to the reactivity of the aldehyde warhead allowing for the reversible formation of a hemiacetal. A similar difference in reactivity is observed for **CAT811** (30nM) and its alcohol analogue (700nM).

4.5: Conclusion

Molecular modelling with the calpain 1 construct model show that macrocyclic alcohols **4.1b** and **4.2b** and aldehydes **4.1c** and **4.2c** bind in the active site in a *β*-strand conformation and with appropriate warhead distance and low negative Glide scores and Emodel scores. On the basis of these results, macrocycles **4.1a-c** and **4.2a-c** were chosen for synthesis and biological evaluation. To develop structure-activity relationships and to investigate the validity of the modelling, macrocycles **4.3a-c** (where modelling suggested they could not bind in a *β*-strand conformation) were also prepared.

We have investigated strategies for the synthesis of macrocycles **4.1-4.3**. An intramolecular nucleophilic substitution methodology, developed for an efficient large

scale synthesis of **CAT811**, was unsuccessful for the preparation of **4.1** as a key intermediate **4.10** could not be isolated and purified. An alternative synthetic route to **4.1** was investigated involving intramolecular lactamization. This methodology proved to be successful for the synthesis of **4.1**, **4.2** and **4.3**. The macrocycles **4.1-4.3** required alkylation on N-1 of the imidazole ring of the histidine derivatives **4.22** and **4.40**. The selective alkylation was confirmed from ^{1}H NMR analysis including Heteronuclear Multiple Bond Correlation (HMBC), HSQC, COSY, etc.

The assay results above demonstrate the value of molecular modelling as a screening mechanism before unproductive synthetic work is considered.

Reference

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[5] Glide Score is a scoring function based on ChemScore and designed to estimate the free energy of binding for the protein–ligand complex. The function uses simple contact terms to estimate lipophilic and, where relevant, metal–ligand binding contributions, a simple explicit form for hydrogen bonds and a term which penalises flexibility. The Emodel is a model energy score that combines energy grid score, binding affinity predicted by GlideScore, and (for flexible docking) the internal strain energy.

[6] *Schrödinger Suite 2006 Induced Fit Docking Protocol*, Glide version 4.0, Prime version

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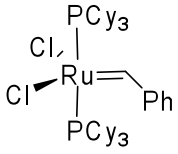
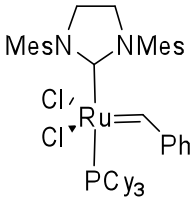
Chapter 5: A study on a water soluble polyethylene glycol immobilized ruthenium catalyst

5.1: Introduction

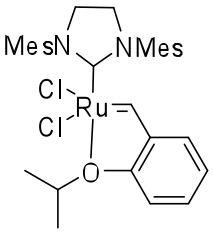
Ruthenium-based catalysts **1.28** and **1.29** are widely used in a range of organic solvent-based olefin metathesis reactions because of their high functional group tolerance and ease of handling (e.g. see section 1.5.1 in Chapter 1). Catalyst **1.29** shows increased stability and activity when compared to **1.28**, which has been attributed to electron donating ability and the steric bulk of the NHC ligand.[1] Hoveyda and co-workers reported a further advancement in catalyst design with the complex **5.1**, where a remaining phosphine ligand is replaced by a chelating ether, further decreasing the air sensitivity of the catalyst and improving catalyst stability.[2]



1.28

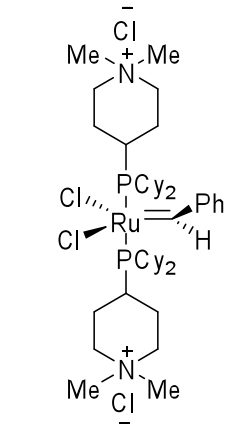


1.29

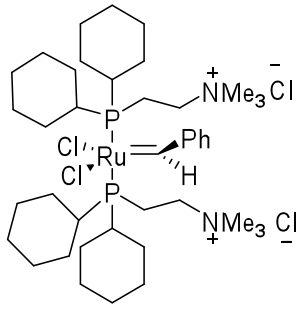


5.1

We considered that alkene metathesis in aqueous media[3,4] would be useful for the preparation of water soluble examples of the macrocyclic-based calpain inhibitors discussed in the previous chapters of this thesis. However, the low water solubility of existing catalysts limits their use under these aqueous conditions. A number of water-soluble catalysts have been reported[5] and these have been used for alkene metathesis in aqueous solution. Catalysts **5.2** and **5.3** were developed by substituting a hydrophobic phosphine in **1.28** with electron-rich, cationic phosphine.[6] These catalysts are completely soluble in water and methanol mixtures and they have been shown to catalyze the ROMP of compounds **5.4** and **5.6** in water, methanol and aqueous emulsions (Scheme 5.1). The catalyst **5.3** is more active for RCM in water and methanol compared to **5.2.**



5.2



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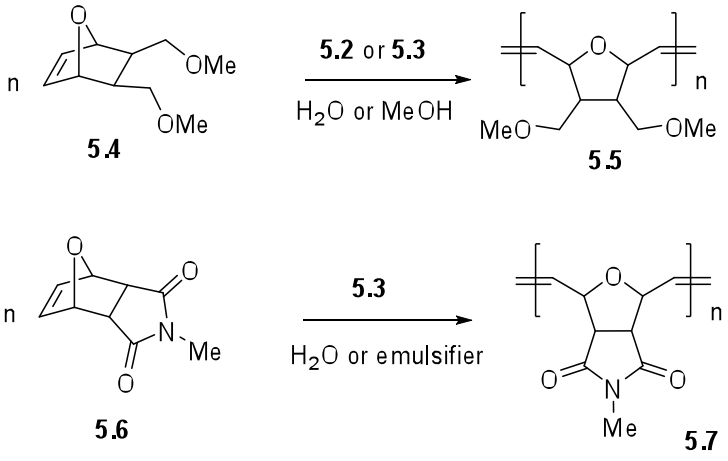
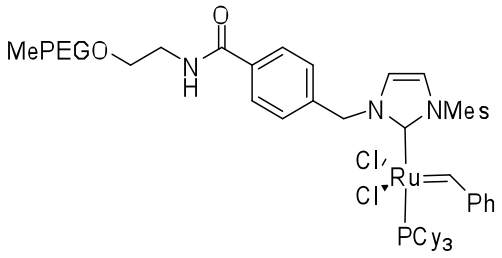


5.4 **5.5** **5.6** **5.7**

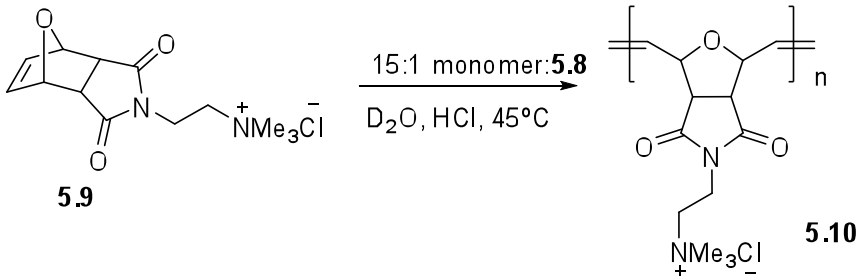
Scheme 5.1: ROMP of compounds **5.4** and **5.6** in water, methanol and aqueous emulsions in the presence of catalyst **5.2** and **5.3**.

Grubbs and coworkers[7] have reported the aqueous soluble polymer-supported catalyst **5.8** which has a polyethyleneglycol (PEG) attached to the *N*-benzyl NHC of catalyst **1.29** rendering it soluble in water and some organic solvents such as dichloromethane and toluene, but insoluble in diethyl ether. Catalyst **5.8** can therefore be recovered and reused from alkene metathesis product by simple extraction from diethyl ether thereby being economically attractive.[8] The ROMP of **5.9** with catalyst **5.8** gave **5.10** in 73% conversion after 24 h. Further conversion was not observed after additional 12 h,

however, reaction in the presence of 1 equiv of HCl gave an improved conversion (95%) after 12 h.



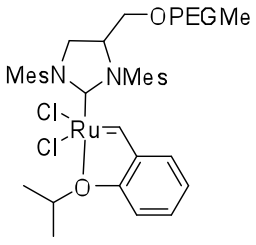
5.8



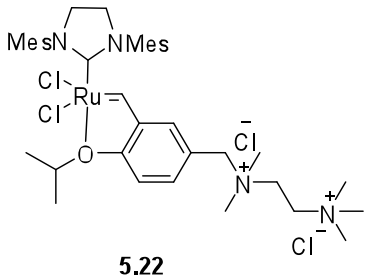
5.9 **5.10**

Scheme 5.2: ROMP of **5.9** with catalyst **5.8**.[7]

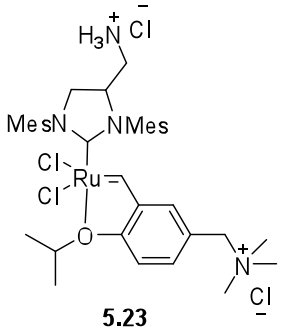
Hong and Grubbs[9] reported **5.11** formed by linking the PEG to the NHC backbone of a Hoveyda-Grubbs type catalyst. This catalyst shows significant activity in catalyzing the RCM in aqueous media. Jordon and Grubbs[10] have also reported small-molecule water-soluble catalysts **5.22** and **5.23** by attaching quaternary ammonium salts to the benzylidene, and NHC ligands respectively. Both catalysts are efficient for RCM, ROMP, and CM in water.



5.11



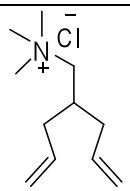
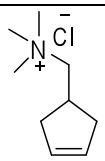
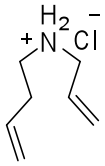
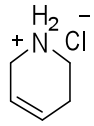
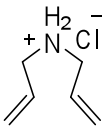
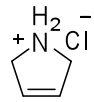
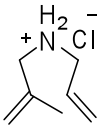
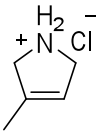
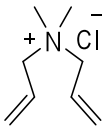
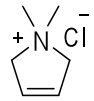
5.22



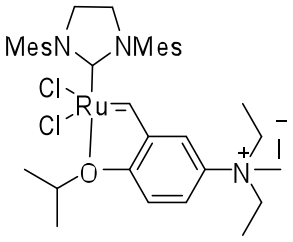
5.23

Grubbs[10] has reported RCM of dienes (as shown in Table 5.1) with catalysts **5.11, 5.22** and **5.23** in water. RCM of dienes **5.12** and **5.14** (Table 5.1) gave 5- and 6-membered ring compounds **5.13** and **5.15** in good yields. Cyclisation of diene **5.16** to **5.17** gave a satisfactory conversion with catalyst **5.22**, but low conversions using **5.11** and **5.23**. RCM of **5.18** with catalysts **5.11, 5.22** and **5.23** gave modest yields of the cyclic product, and RCM of 5.20 with 5.11, 5.22 and 5.23 give poor yields

Table 5.1. RCM reactions in water.

Catalysts	Substrate	Product	Time (h)	Conversion (%)
5.11			12	>95%
5.22			24	>95%
5.23	**5.12**	**5.13**	0.5	>95%
5.11			24	>95%
5.22			24	>95%
5.23	**5.14**	**5.15**	4	84%
5.11			36	67%
5.22			24	>95%
5.23	**5.16**	**5.17**	6	36%
5.11			24	42%
5.22			24	70%
5.23	**5.18**	**5.19**	4	26%
5.11			24	<5%
5.22			24	<5%
5.23	**5.20**	**5.21**	24	<5%

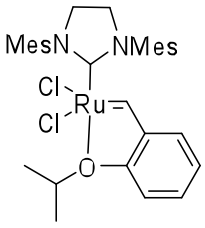
Grela and Connon [11, 12] also reported the diethylmethylammonium-substituted catalyst **5.24** which shows moderate solubility in water, with similar activity to **5.22** and **5.23** for RCM and CM. Catalyst **5.24** also mediates RCM of dienes in homogeneous water/alcohol mixtures to give macrocycles in good and excellent yields. The high activity of catalyst **5.24** is ascribed to the electron-withdrawing ammonium group that enhances the catalyst initiation rates.



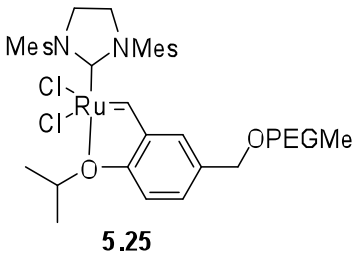
5.24

5.2: Studies on a new water soluble metathesis catalyst

Work in the Abell group by Dr Shazia Zaman recently lead to development of the complex **5.25**, which constitutes immobilization of Hoveyda-Grubbs catalyst **5.1** on a water soluble polyethylene glycol (PEG).[5] This catalyst has been shown to have activity in RCM of various dienes in dichloromethane in air and an ability to be recycled. [13] For example, RCM of diene **5.26** under reflux in the presence of 10% catalyst **5.25** gave olefin**5.27** in near quantitative conversion after 1 h. The addition of diethyl ether to the final reaction mixture precipitates the catalyst which can be filtrated and reused in the subsequent RCM. This sequence was repeated a further three times without significant loss in catalytic activity (see Table 5.2).

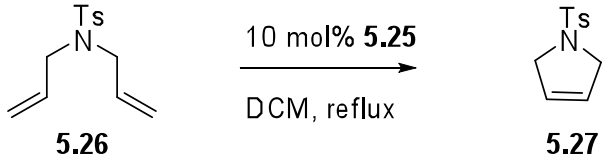


5.1



5.25

Table 5.2. Recyclability studies of catalyst **5.25** in RCM of diene **5.26**.

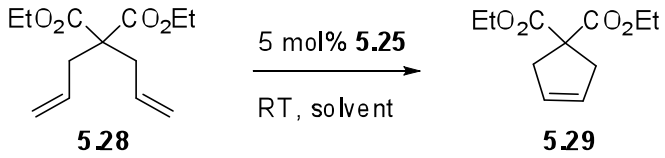


5.26 **5.27**

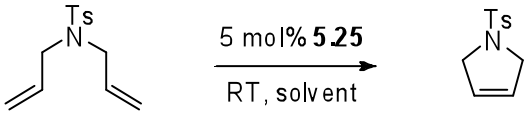
Cycle	Conversion (%)
1	>98
2	95
3	95
4	90
5	89

We recently reported the results of using this catalyst **5.25** to promote RCM reactions of a range of dienes in water-organic solvent media.[14] First, organic solvents were screened as co-solvent for RCM using catalyst **5.25** in aqueous media. RCM of the dienes **5.28** and **5.30** were investigated using **5.25** in methanol/water, ethanol/water and acetone/water, in order to establish catalyst efficiency and to develop optimum conditions (Table 5.3). The dienes **5.28** and **5.30** were chosen as model compounds as they have been used in related studies.[15] These dienes were then treated with 5 mol% of catalyst **5.25** at rt in the specified solvent system as shown in Table 5.3. Conversion to **5.29** and **5.31** was monitored after both 1 h and 16 h by ^{1}H NMR analysis of the crude reaction.

Table 5.3. Studies of catalyst **5.25** in different aqueous systems in the RCM of diene **5.28** (entry 1-3) and **5.30** (entry 4-6).



5.28 **5.29**



5.30 **5.31**

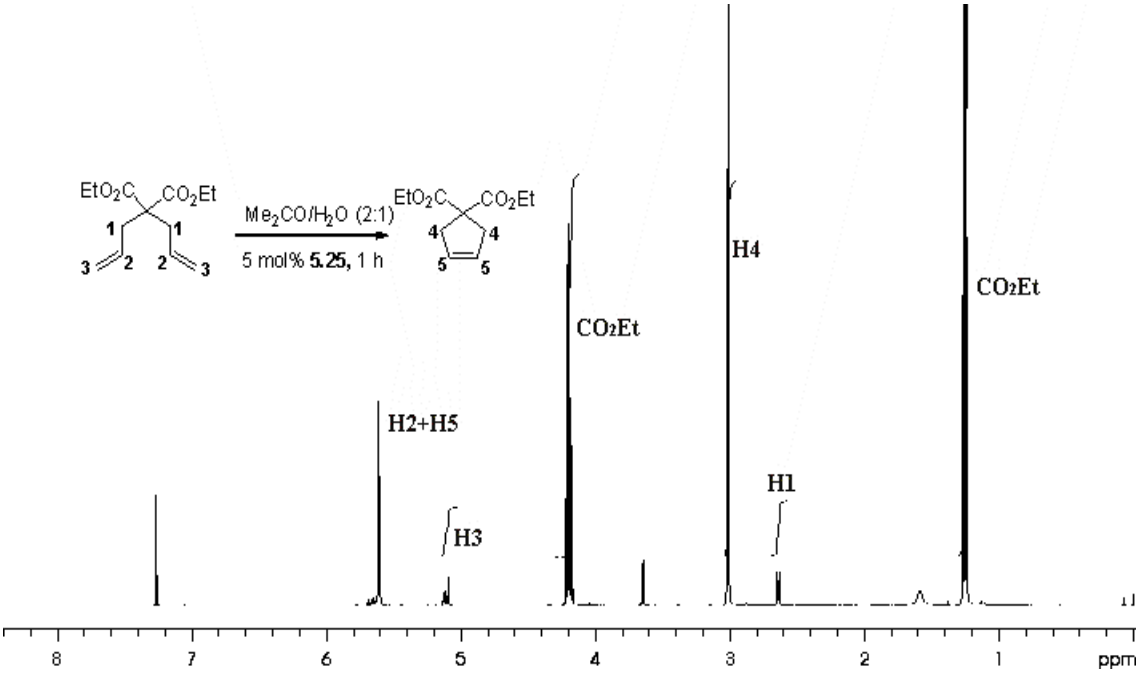
Entry	Solvent (2/1)	Time (h)	Conversion (%)[a]
1	Acetone/H_2O	1	73%
		16	95%
2	MeOH/H_2O	1	42%
		16	50%
3	EtOH/H_2O	1	57%
		16	72%
4	Acetone/H_2O	1	98%
		16	98%
5	MeOH/H_2O	1	88%
		16	91%
6	EtOH/H_2O	1	93%
		16	94%

[a] Determined by analysis of the crude ^{1}H NMR spectrum**.**

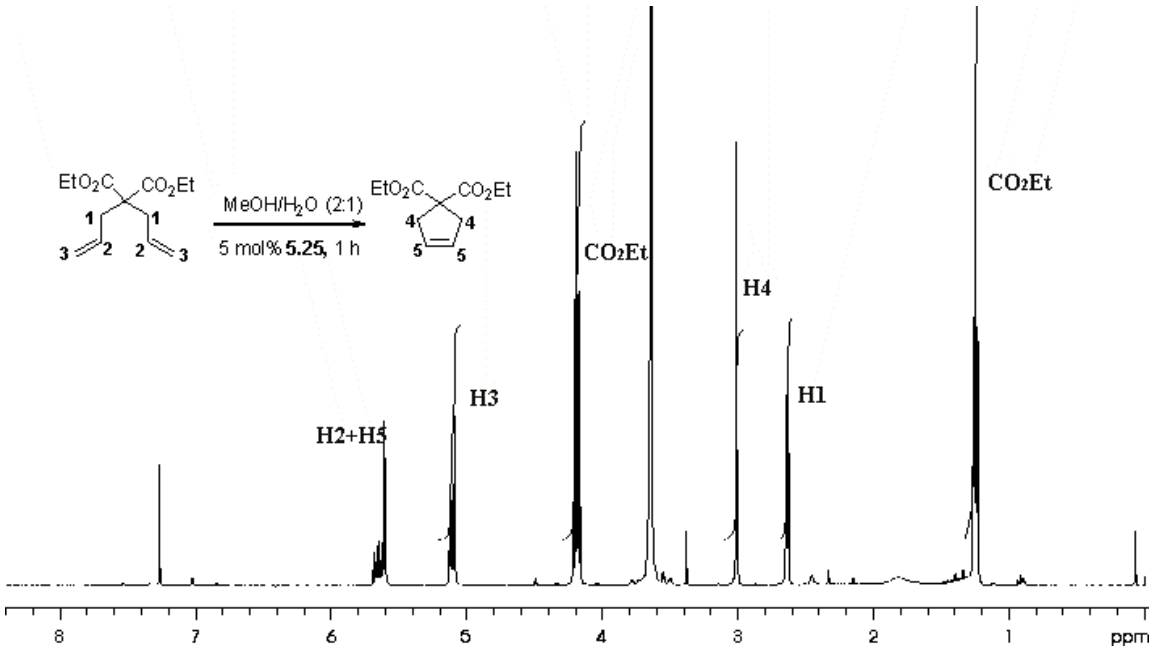
The results in Table 5.3 show acetone/water (2:1, v/v) is a good solvent system. Reaction of **5.28**, in solvents acetone/water, methanol/water, ethanol/water, gave 73%, 42% and 57% conversions to **5.29** respectively after 1 h which increased to 95%, 50% and 72% after 16 h (entry 1-3, Table 5.3).

The conversion percentages were established by ^{1}H NMR analysis of resonances in both diene **5.28** and cyclic alkene**5.29** (Figure 5.1). For example, the ^{1}H NMR spectrum for

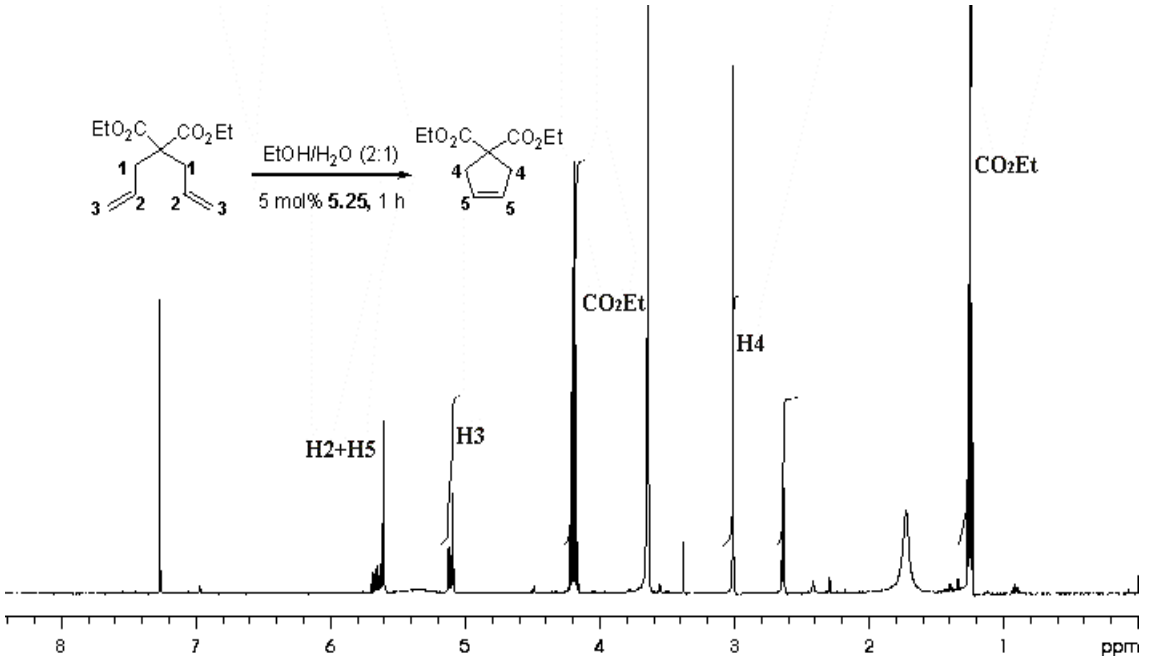
the reaction mixture in acetone/water after 1 h (Figure 5.1a) showed the presence of two resonances centered at 2.62 and 3.01 ppm, in the ratio of 1:2.7, corresponding to methylene protons H4 and H1 in diene **5.28** and cyclic alkene **5.29** respectively. The ratio of 1:2.7 represents the molar ratio of **5.28** to **5.29** in the crude and the reaction therefore gave an approximately 73% conversion.



(a)



(b)



(c)

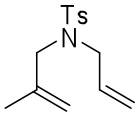
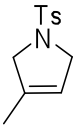
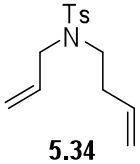
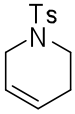
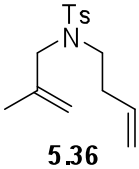
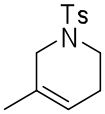
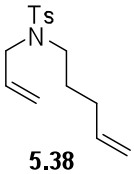
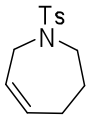
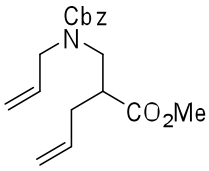
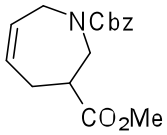
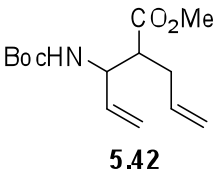
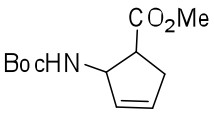
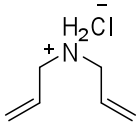
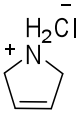
Figure 5.1. Investigation of the solvent systems for RCM of **5.28** by ^{1}NMR analysis.

RCM of diene **5.30** was also investigated in solvents acetone/water, methanol/water, and ethanol/water, respectively. Similarly acetone/water solution also gave the best conversion of diene **5.30** to **5.31** (98%) which compares to methanol/water and ethanol/water systems with 91% and 94% conversion respectively (see entry 4-6, Table 5.3).

The efficiency of catalyst **5.25** for RCM reactions with a range of alkenes in acetone/water solution was then investigated. The substrates were prepared by Dr Shazia Zaman for the study of RCM reactions (Table 5.4). The *N*-tosyl based-dienes **5.32**, **5.34**, **5.36**, **5.38**, *β*-amino acid derived dienes (**5.40** and **5.42)** and the water-soluble ammonium chloride based diene **5.44** were synthesized by Dr Zaman according to literature procedures.[16,17]

The ^{1}H NMR spectra of the RCM products **5.33**, **5.35**, **5.37**, **5.39**, **5.41**, **5.43** and **5.45** were compared with previously reported[16,17,18,19] structures. The conversion to RCM products was evaluated by ^{1}H NMR spectroscopy after both 1 h and 16 h reaction times (Table 5.4). All the reactions were carried out with 0.2 M of dienes in acetone and water solvent system (2:1) in the presence of 5 mol% of catalyst **5.25**. The reaction mixture was stirred at rt without the exclusion of air.

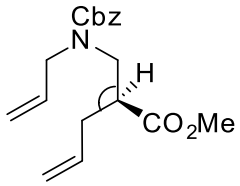
Table 5.4. Ring closing metathesis of substituted olefins in acetone and water.

Entry	Substrates	Product	Time (h)	Conversion (%)[a]
1	 **5.32**	 **5.33**	1 16	98% 98%
2	 **5.34**	 **5.35**	1 16	95% 98%
3	 **5.36**	 **5.37**	1 16	72% 83%
4	 **5.38**	 **5.39**	1 16	52% 71%
5	 **5.40**	 **5.41**	1 16	90% 98%
6	 **5.42**	 **5.43**	1 16	67% 72%
7	 **5.44**	 **5.45**	1 16	<5% 35%

The catalyst shows significant efficiency in the ring-closing metathesis reaction to form both 5- and 6-membered N-containing heterocycles. For example, RCM of *N*-tosyldiallyl dienes **5.32** and **5.34** with catalyst **5.25** gave the 5- and 6-membered heterocycles **5.33** and **5.35** in almost quantitative conversion after 1 h (Table 5.4, entries 1-2). Reaction of gem-disubstituted alkene **5.36** results in conversion to **5.37** in 72% after 1 h and 83% after 16 h (Table 5.4, entry 3).

Catalyst **5.25** proved less efficient at forming a 7-membered heterocycle compared with 5- and 6-membered rings. RCM of **5.38**, under the above conditions, resulted in only 52% conversion to the 7-membered heterocycle **5.39** after 1 h. The conversion was increased to 71% after an extended 16h reaction.

It appears that introducing a methyl ester substitution (not on the alkene) to the acyclic precursor diene, as in **5.40** (Figure 5.2), significantly enhances conversion to the heterocycle, with 90% conversion to **5.41** after only 1 h reaction. This observation is consistent with the well-known Thorpe Ingold effect[20] which states that substitutions on acyclic precursors compress the internal angle (θ) of carbons in the open-chain structure, leading to the two reactive terminal groups closer to each other, thereby facilitating ring closure. The ester substitution on diene **5.40** (see Figure 5.2) results in a reduced internal angle (θ) of carbons in acyclic structure. This brings two terminal alkenes closer together and facilitates RCM of **5.40** to **5.41**.



5.40

Figure 5.2. Thorpe Ingold effect on diene **5.40**.

The diene **5.42**, which lacks an amine, underwent cyclisation less efficiently to give the 5-memberred compound **5.43** in only 67% yield after 1 h (see entry 6, Table 2). Finally, RCM of the water soluble-diene substrate **5.44** in the presence of catalyst **5.25** resulted in a 35% conversion to the 5-membered heterocycle **5.45** after an extended 16 h reaction time (entry 7, Table 5.4).

5.3: Conclusion

In summary, catalyst **5.25** with a water soluble polyethylene glycol chain (PEG) positioned in Hoveyda-Grubbs catalyst **5.1** shows, like the Grubbs compound, RCM activity in aqueous solvent mixtures (acetone/water, ethanol/water, methanol/water: 2:1, v/v) with acetone/water being best. This catalyst **5.25** and solvent system, namely acetone/water, could be explored for the preparation of water soluble versions of the macrocyclic protease inhibitors discussed in this thesis and this work is currently underway in Adelaide.

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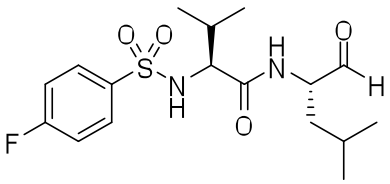
Chapter 6: Study of the importance or otherwise of a fluorine-H-bond for SJA-6017 and its alcohol precursor in the S3 pocket of cysteine proteases

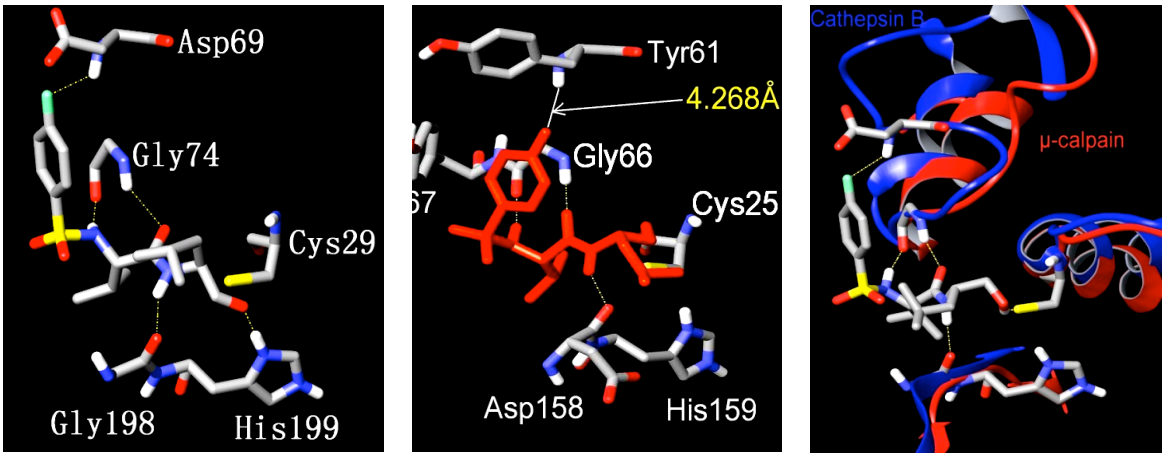
6.1: Introduction

SJA-6017 has been reported by Senju Pharmaceuticals Ltd[1] to be a potent calpain 1 inhibitor and is effective in preventing calcium-induced cortical cataract in rats (see Chapter 1). The inhibitory activity of **SJA-6017** on two other cysteine proteases namely papain and cathepsin B shows that **SJA-6017** is more potent toward cathepsin B (22 nM) than to calpain 1 (130 nM) and papain (23 nM) (Personal communication from Dr Jim Morton at the University of Lincoln).



SJA-6017

This difference of inhibitory activity of **SJA-6017** was consistent with the computer docking studies by Wanting Jiao which showed compound **SJA-6017** in the active sites of cathepsin B, calpain 1 and papain exhibited three hydrogen bonds between the peptide backbone and the enzyme active site residues, namely Gly66/Gly74/Gly208 and Asp158/Gly198/Gly271 respectively (Figure 6.1). These three hydrogen bonds are crucial for stabilisation of an inhibitor into a β-strand, a conformation that is generally recognised by active sites of proteases (see section 1.4, introduction).[9] However, there was an additional H-bond formed between the fluorine of fluorobenzyl ring of **SJA-6017** and the NH group of Asp69 in the S3 pocket of cathepsin B (Figure 6.1a). This F-H bond was not observed for **SJA-6017** when docked with papain and calpain 1. The extra H-bond formed between **SJA-6017** and cathepsin B would be expected to further stabilise the β-strand conformation of this compound in the active site resulting in a higher potency towards cathepsin B than to calpain and papain.



(a) (b) (c)

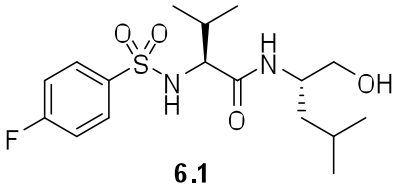
Figure 6.1: Modelling results for Compounds **SJA-6017** in active sites of (a) cathepsin (b) papain and (c) calpain 1. All structures are displayed in element colour scheme, where oxygen is in red, nitrogen in blue, sulphur in yellow, hydrogen in white, fluorine in green, and carbon in grey. H-bonding interactions are shown by yellow dashed lines.

The F-hydrogen bond was not observed in modelling results for **SJA-6017** in papain because the large tyrosine side chain 61 at the active site of papain is located at the same position as the hydrogen bonding Asp69 in cathepsin B (Figure 6.1b). The tyrosine side chain block with the fluorobenzyl ring of **SJA-6017** such that the F atom is 4.268Å from the NH group of Tyr61, a distance too great for the formation a F-H bond.

For calpain 1, the loop of cathepsin B that contains the H-bonding Asp69 residue to the fluorine atom is not present (Figure 6.1c) and therefore formation of the F-hydrogen bond at this position is not observed thereby accounting for the selectivity of **SJA-6017** towards cathepsin B as observed in the assay results.

The assay results of the fluorobenzylsulfonyl alcohol **6.1**, the synthetic precursor of **SJA-6017**, was also selective for cathepsin B (9.2 μM) over calpain 1 (50 μM) and papain (> 50 μM). These assay results are consistent with the molecular modelling studies which

show that an extra F-hydrogen bond was observed in the active site of cathepsin but not in papain and calpain 1.



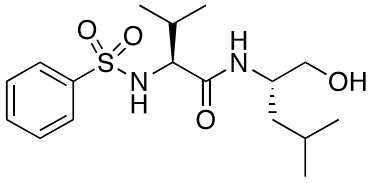
6.1

The alcohol **6.1** proved to be relatively more selective for cathepsin B than its corresponding aldehyde **SJA-6017** (Table 6.1). For an inhibitor with a reactive warhead such as an aldehyde, the effect of the extra F-H interaction at the P3 position of the inhibitor will not be as important as for the alcohol inhibitors where bonding to the warhead is weaker and interaction relies on non-covalent interactions. Therefore, an extra F-hydrogen bond has a greater impact on the relative activity of the alcohols compared to the aldehydes.

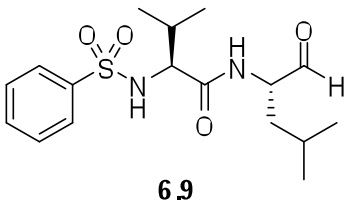
Table 6.1

Compound	IC_{50} (μM)		
	papain	cathepsin B	calpain 1
SJA-6017	0.023	0.022	0.13
6.1	>50	9.2	50

The results above led us to propose that the fluoro group of **6.1** and **SJA-6017** may be important in forming a H-bond with the S3 pocket of the active site of cathepsin B. As such, removal of the fluoro group of **SJA-6017** will be expected to result in a compound (**6.9**) that is less selective than **SJA-6017** to cathepsin B. To investigate the validity of the modelling and develop a structure-activity relationship we prepared **6.8** and **6.9** as des-fluoro analogues of **6.1** and **SJA-6017**, respectively.



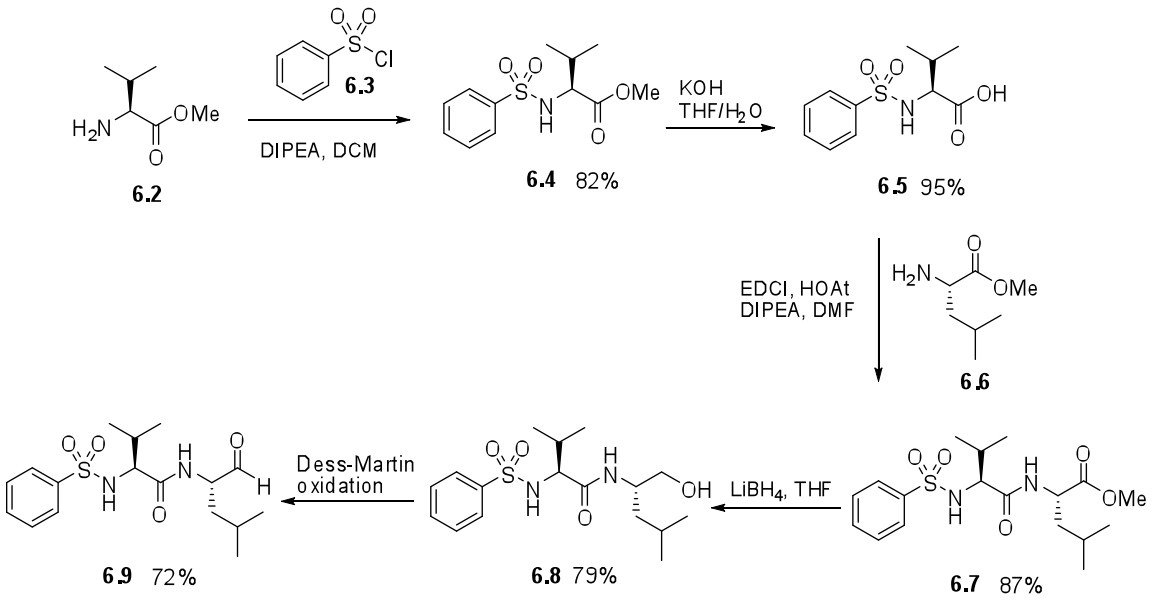
6.8



6.9

6.2: Synthesis of alcohol 6.8 and aldehyde 6.9

The synthetic route to dipeptide alcohol **6.8** and aldehyde **6.9** is shown in Scheme 6.1. Commercially available Val-OMe **6.2** was reacted with benzene sulfonyl chloride **6.3** to give **6.4** in an 82% yield. Hydrolysis of the ester **6.4** under basic conditions gave carboxylic acid **6.5** (95%). Coupling of **6.5** with the commercially available Leu-OMe **6.6** in the present of EDCI and HOAt gave the dipeptide **6.7** (87%). The molecular formula of **6.7** was confirmed by a parent ion at m/z in mass spectrum and ^{1}H NMR spectrum of this compound shows two resonances at 3.62 ppm and 4.32 ppm corresponding to α-protons of valine and leucine. The ester group of **6.7** was reduced with $LiBH_4$ to give alcohol **6.8** in 79%. Freshly prepared Dess-Martin periodinane[2] was reacted with **6.8** to give aldehyde **6.9** (72%).



Scheme 6.1. Synthesis of alcohol **6.8** and aldehyde **6.9**.

Samples of these two compounds have been submitted to Dr Jim Morton at Lincoln University for biological assay but the earthquake has delayed this work.

References

[1] Inoue, J.; Nakamura, M.; Cui, Y.; Sakai, Y.; Sakai, O.; Hill, J. R.; Wang, K. K. W.; Yuen, P. *J. Med. Chem.,* **2003**, *46*, 868-871.

[2] Boeckman, R. K.; Shao, P.; Mullins, J. J. *Organic Syntheses, Coll*., **2004**, *10*, 696; **2000**, *77*, 141.

Chapter 7: Experimental

7.1: General Experimental Methods

^{1}H NMR spectra were recorded on an Inova 500 spectrometer operating at 500 MHz unless otherwise stated. Carbon NMR spectra were obtained on a Varian Unity XL 300 MHz Fourier Transform spectrometer operating at 75 MHz. Chemical shifts are reported in parts per million (ppm, δ) referenced relative to the residual solvent peak; coupling constants are reported in Hz. Electrospray ionization (ESI) mass spectra were determined on a micromass liquid chromatography time of flight mass spectrometer, with a probe voltage of 3200 V, temperature of 150 ^{o}C and a source temperature of 80 ^{o}C. Direct ionization used 10 μL of a 10 μgmL^{-1} solution, using a carrier solvent of 50% acetonitrile/water at a flow rate of 20μL min^{-1}. Ionization was assisted by the addition of 0.5% formic acid. Melting points were obtained on an electrothermal melting point apparatus and are uncalibrated. TLC was performed on aluminium-backed Merck Kieselgel KG60F254 silica plates. Amino acid starting materials were purchased from GL Biochem (Shanghai) Limited. Reagents and other chemicals were purchased from Aldrich Chemicals.

General procedure A1: 1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide hydro chloride (EDCI) mediated peptide coupling reaction.

To a stirred solution of the *N*-protected amino acid derivative (1.0 equiv) in dry DMF (0.1-0.5 M) was added amine (1.1 equiv), DIPEA (2.4 equiv), EDCI (1.2 equiv) and HOAt (1.2 equiv). The solution was stirred at rt overnight, diluted with ethyl acetate and the organic layer washed twice with aqueous HCl (1 M), saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$ and the solvent removed *in vacuo*.

General Procedure A2: O-(7-azabenzotriazole-1-yl)-*N,N,N',N'*-tetramethyluronium hexa fluorophosphate (HATU) mediated peptide coupling reaction.

To a stirred solution of the *N*-protected amino acid derivative (1.0 equiv) in dry DMF (0.1-0.5 M) was added amine (1.1 equiv), DIPEA (2.4 equiv) and HATU (1.2 equiv). The

solution was stirred at rt overnight, diluted with ethyl acetate and the organic layer washed twice with aqueous HCl (1M), saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$ and the solvent removed *in vacuo*.

General procedure B: Ester hydrolysis with base.

To a solution of the ester in methanol was added NaOH (4 equiv) pre-dissolved in water. The solution was heated to 65^{o}C with stirring overnight. The aqueous layer was acidified to pH 2 with 1M HCl and the product extracted twice with ethyl acetate. The combined organic layers were washed with water, brine, and dried over $MgSO_4$ and the solvent was removed in vacuo.

General procedure C1: Ring closing metathesis under thermal reflux.

To a solution of diene in anhydrous 1,1,2-trichloroethane (0.01 M) under an inert atmosphere was added Grubb's second generation catalyst (10 mol %) and the mixture heated under reflux. After 1 h a second portion of catalyst (10 mol %) was added, and the mixture was heated for 1 h before the final portion (10 mol %) was added. The reaction mixture was heated under reflux for a further 16 h, cooled, stirred overnight with activated charcoal, filtered, and the solvent was removed *in* v*acuo*.

General procedure C2: Ring closing metathesis under microwave reflux.

To a solution of diene in anhydrous 1,1,2-trichloroethane (0.01 M) under an inert atmosphere was added Grubb's second generation catalyst (10 mol %), and the reaction mixture was heated for 20 min in a microwave (1200 W, 110-115 °C). Two further portions of catalyst (2 × 10 mol %) were added with 20 min heating between each addition. The reaction mixture was cooled, stirred overnight with activated charcoal, filtered, and the solvent removed *in* v*acuo*.

General procedure C3: Ring closing metathesis under thermal condition with chlorodicyclohexylborane.

To a solution of diene in anhydrous 1,1,2-trichloroethane (0.01 M) under an inert atmosphere was added Grubb's second generation catalyst (10 mol %) and chlorodicyclo-

hexylborane (1 M solution in hexane, 10 mol %). After 1 h a second portion of catalyst (10 mol %) was added, and the mixture was heated for 1 h before the final portion (10 mol %) was added. The reaction mixture was heated under reflux for a further 16 h, cooled, stirred overnight with activated charcoal, filtered, and the solvent was removed *in* v*acuo*.

General procedure C4: Ring closing metathesis under microwave reflux with chlorodicyclohexylborane.

To a solution of diene in anhydrous 1,1,2-trichloroethane (0.01 M) under an inert atmosphere was added Grubb's second generation catalyst (10 mol %) and chlorodicyclohexyl borane (1 M solution in hexane, 10 mol %). The reaction mixture was then heated for 20 min in a microwave (1200 W, 110-115^{o}C). Two further portions of catalyst (2 × 10 mol %) were added with 20 min heating between each addition. The reaction mixture was cooled, stirred overnight with activated charcoal, filtered, and the solvent removed *in* v*acuo*.

General procedure C5: Ring closing metathesis under thermal condition with titanium-based Lewis acid.

To a solution of diene in anhydrous 1,1,2-trichloroethane (0.01 M) under an inert atmosphere was added Grubb's second generation catalyst (10 mol%) and $Ti(O_iPr)_4$ (0.1 equiv). After 1 h a second portion of catalyst (10 mol %) was added, and the mixture was heated for 1 h before the final portion (10 mol %) was added. The reaction mixture was heated under reflux for a further 16 h, cooled, stirred overnight with activated charcoal, filtered, and the solvent was removed *in vacuo*.

General procedure C6: Ring closing metathesis in aqueous media.

A soluble polyethylene glycol immobilized ruthenium catalyst (0.05 equiv) was added to a 1 ml vial, equipped with a magnetic stirring-bar and sealed with a septa-cap. The vial was then charged with a 0.2 M solution of diene in aqueous media (2:1, v/v). The reaction was stirred at rt without exclusion of air. The progress of the reaction was analyzed by ^{1}H NMR after 1 h and 16 h for all the reactions studied.

General procedure D: Hydrogenation of a carbon-carbon double bond.

The alkene was dissolved in a 1:1 mixture of methanol and ethyl acetate and 20% (w/w) of 10% palladium on carbon catalyst was added. The mixture was stirred vigorously in an atmosphere of hydrogen at rt for 18 h. The mixture was filtered through celite and concentrated *in-vacuo*.

General procedure E1: *N*-Boc deprotection.

Acetyl chloride was added dropwise to an ice-cooled solution of dry methanol and stirred for 20 minutes before adding the *N*-Boc protected compound. The reaction solution was stirred overnight and concentrated *in vacuo*.

General procedure E2: *N*-Boc deprotection.

To a solution of *N*-Boc protected amino acid derivative in methanol (0.01 M) cooled in ice bath, thionyl chloride (1.2 equiv) was added drop wise. The solution was stirred on an ice bath for 1 h and allowed to warm to rt and stirred for 18 h. The solvent was removed *in vacuo*.

General procedure E3: *N*-Boc deprotection.

N-Boc protected amino acid derivative was dissolved in dichloromethane (0.05 M) and trifluoroacetic acid (30 ml) was added drop wise. The solution was stirred under the nitrogen atmosphere at rt for 3 hours. The solvent was removed in *vacuo* and residue was dissolved in toluene, and the solution re-evaporated to give the product without purification.

General Procedure F: Cbz *N*-terminal protection.

To amine dissolved in anhydrous DMF was added benzyl chloroformate (1.5 equiv) and DIPEA (4.0 equiv) and the mixture stirred at rt for 18 h. The reaction mixture was partitioned between ethyl acetate and aqueous HCl (1 M). The aqueous phase was extracted twice with ethyl acetate and the combined organic extracts were washed with brine, dried over $MgSO_4$ and the solvent was removed *in vacuo*.

General Procedure G1: Lithium aluminium hydride reduction of methyl ester.

A solution of the methyl ester in anhydrous THF (0.1 M) under a nitrogen atmosphere was cooled on an ice bath. $LiAlH_4$ (1.1 equiv) in THF (1 M) was added and the mixture stirred in ice for 1 h and allowed to warm to rt and stirred for a further 16 h. Methanol was added drop wise with stirring. The solvent was removed *in vacuo* and the residue partitioned between ethyl acetate and aqueous $KHSO_4$ (1 M). The aqueous phase was extracted twice with chloroform and the combined organic phase was washed with brine, dried over $MgSO_4$ and the solvent removed *in vacuo*.

General Procedure G2: Lithium borohydride reduction of methyl ester.

To a suspension of macrocyclic methyl ester (1 equiv) in dry THF were added methanol (2 equiv) and solution of $LiBH_4$ (2 equiv) in THF (2.0 M). The resulting mixture was stirred overnight at rt and then THF evaporated *in vacuo* washed with aq. HCl (1 M) and ethanol. The organic phase was separated, further washed with water, dried over $MgSO_4$, and the solvent removed *in vacuo*.

General Procedure H1: SO_3.pyridine oxidation.

The macrocyclic alcohol (1 equiv) was dissolved in a mixture of dichloromethane and DMSO (2:1 v/v) and the solution cooled in an ice-bath. DIPEA (4 equiv) was added and the solution was stirred for 5 min before the addition of a warmed solution (approx 40 ^oC) of SO_3.pyridine complex (4 equiv) in DMSO. The reaction mixture was stirred for 3 h, allowed to warm to rt, and then it was diluted with ethyl acetate. The organic phase was separated and then washed successively with aq. HCl (1 M), sat. aq. $NaHCO_3$, and brine, dried over $MgSO_4$ and the solvent was removed *in vacuo.*

General Procedure H2: Oxidation of an alcohol using SO_3.Py complex with a sacrificial isopropyl alcohol.

Under nitrogen, the alcohol (x equiv, where x < 373 µmol) and isopropyl alcohol (373-x equiv, so total of moles alcohol and isopropyl alcohol is 373 µmol) were dissolved in DMSO (4 ml). To this dichloromethane (2 ml) was added and the solution cooled on an ice bath. While stirring under nitrogen, DIPEA (4 equiv., 1.49 mmol, 260 µL) was added.

To this a solution of SO_3.Py complex (4 equiv, 1.49 mmol) dissolved in DMSO (1.5 ml) was added drop wise over 5 minutes. The reaction mixture was left stirring for 3 hours while allowing to warm to rt. The reaction mixture was then diluted with ethyl acetate (40 ml), washed with HCl (1M, 2 x 30 ml), sat. $NaHCO_3$ (2 x 30 ml) and brine (2 x 30 ml). The organic layer was dried over $MgSO_4$ and the solvent was removed *in vacuo*.

General Procedure H3: Dess-Martin oxidation.

Alcohol (1.0 equiv) was suspended in anhydrous dichloromethane (0.1 M) under a nitrogen atmosphere. Dess-Martin reagent (3.0 equiv) was added to the solution with vigorous stirring under a nitrogen atmosphere. The reaction mixture was stirred for 1 h at rt and was then quenched by adding saturated sodium bicarbonate solution containing 10 % of $Na_2S_2O_5$. The aqueous layer was extracted with dichloromethane and the combined organic phase was washed with water and dried with brine and sodium sulphate and solvent evaporated *in vacuo*.

General Procedure I1: Diazotization.

To a suspension of *N*-Boc-Lys-OH (1 equiv) in water (0.2 M) at 60 °C was added 4M NaOH (2 equiv). Sodium nitroprusside (1.5 equiv) was added in portions over 1 h, while maintaining the pH at 9.5 by the addition of 4 M NaOH as required. After the nitroprusside was completely added, the resulting red suspension was stirred for a further 6 h at 60 °C. The resulting suspension was cooled to 10 °C, and 1 M HCl added until a pH of 1 was achieved. The resulting solution was extracted with ethyl acetate, the combined organic fractions were dried over $MgSO_4$, and solvent removed *in vacuo*.

General Procedure I2: Diazotization

A solution of *N*-Cbz-Lys-O^{t}Bu (1 equiv) in aqueous AcOH (50% v/v) was treated with $NaNO_2$ (11 equiv) and the resulting mixture was stirred at rt for 4 h. The mixture was then made basic by the addition of aqueous NaOH (40%) and extracted with diethyl ether. The organic layer was treated with a methanolic solution of KOH (0.1 M) for 2 h in order to complete hydrolysis of the formed acetyl derivate of the alcohol. The reaction mixture was then concentrated under reduced pressure, diluted with water and extracted twice

with ethyl acetate. The combined organic fractions were dried over $MgSO_4$, and solvent was removed *in vacuo*.

General Procedure J1: Methylation of carboxylic acid.

Potassium carbonate (1.2 equiv) was dissolved in anhydrous DMF. To this solution was added carboxylic acid (1 equiv) followed by dropwise addition of methyl iodide (1.2 equiv). The reaction mixture was stirred at rt overnight. The mixture was diluted by ethyl acetate, washed by brine and the organic layer concentrated in *vacuo.*

General Procedure J2: Methylation of carboxylic acid.

The carboxylic acid (1 equiv) was suspended in methanol (1 M) and cooled on an ice bath. Thionyl chloride (1.2 equiv) was added dropwise and the solution was stirred on ice bath for 1 hour and at rt overnight. The reaction solution was concentrated *in vacuo* to give crude product.

General Procedure K1: *N*,*N*'-Dicyclohexylcarbodiimide (DCC) mediated *t*-butyl esterification of carboxylic acid.

A solution of the *N*-protected amino acid (1 equiv), 4-(dimethylamino)pyridine (DMAP) (0.1 equiv), and the *t*-butyl alcohol (5 equiv) in dichloromethane (0.2 M) was cooled with stirring in an ice bath. DCC (1.1 equiv) was added, and the reaction mixture was stirred in ice for 2 h and at rt overnight. The solvent was removed *in vacuo*, and the residue was taken up in ethyl acetate and water. The organic layer was separated, washed twice with saturated $NaHCO_3$ and water, and dried over $MgSO_4$ and the solvent removed *in vacuo*.

General Procedure K2: l-Ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydro chloride (EDCI) mediated *t*-butylation of carboxylic acid.

A solution of the *N*-protected amino acid (1 equiv), 4-(dimethylamino)pyridine (DMAP) (0.5 equiv), and *t*-butyl alcohol (1.1 equiv) in dichloromethane (0.2 M) was cooled with stirring in an ice bath. EDCI (1.2 equiv) was added, and the reaction mixture was stirred in ice for 2 h and at rt overnight. The solution was concentrated to dryness *in vacuo*, and the residue was taken up in ethyl acetate and water. The organic layer was separated,

washed twice with saturated $NaHCO_3$ and water, and dried over $MgSO_4$. The solvent was removed *in vacuo*.

General Procedure K3: *t*-Butylation of carboxylic acid

An *N*-protected amino acid derivative (1 equiv) was dissolved in dimethylacetamide (0.1 M) in the presence of benzyltriethylammonium chloride (BTEAC) (1 equiv). Dried potassium carbonate (26 equiv) was then added, followed by *t*-butyl bromide (48 equiv) and the mixture was stirred at 55^{o}C for 24 hours. After cooling, cold water was added to the reaction mixture, and the resulting solid precipitate was filtered and washed several times with water. The organic layer was separated, washed with water, dried over sodium sulfate and concentrated *in vacuo*.

General Procedure L1: Iodination of primary alcohol.

Triphenylphosine (1.5 equiv), imidazole (1.6 equiv) and iodine (1.5 equiv) were added sequentially to a solution of alcohol dissolved in THF (0.5 M) at rt under nitrogen atmosphere. The reaction mixture was stirred overnight and concentrated *in vacuo*.

General Procedure L2: Iodination of primary alcohol.

To a solution of triphenylphosphine (1.5 equiv) in dry dichloromethane under a nitrogen atmosphere was added recrystallised DDQ (1.5 equiv). The resulting red solution was treated with TBAI (1.5 equiv). After stirring for 5 mins, alcohol (1 equiv) was added and reaction was stirred overnight under an inert atmosphere. The solution was diluted with ethyl acetate, washed with saturated $NaHCO_3$ until the aqueous layer was no longer yellow, washed with distilled water, dried over $MgSO_4$ and filtered, and the solvent removed *in vacuo.*

General Procedure M1: Alkylation of tyrosine hydroxyl with dibromobutane.

Alcohol (1 equiv), K_2CO_3 (1.5 equiv) and Cs_2CO_3 (0.1 equiv) were dissolved in acetonitrite. To 1,4-Dibromobutane (10 equiv) was added and the solution heated at reflux overnight. The solution was concentrated *in vacuo* and residue was partitionated

between ethyl acetate and 1M aq.HCl. The organic layer was washed successively with 1M HCl, brine, dried over $MgSO_4$ and concentrated *in vacuo*.

General Procedure M2: Specific alkylation at the N-1 position of imidazole.

Sodium hydride (60% suspension, 3 equiv) was washed with dry hexanes three times and dried under vacuum. *N*-protected histidine derivative (1 equiv) in DMF was added under a nitrogen atmosphere at -15^{o}C. The reaction mixture was stirred for 30 min at -15^{o}C, and alkyl halide (2 equiv) added. The temperature of the reaction was raised to -5^{o}C and the reaction mixture stirred for 4 h under a nitrogen atmosphere. The reaction was quenched by the addition of methanol and the solvent removed under reduced pressure. The residue was extracted with chloroform and dried over $MgSO_4$ and the solvent was removed *in vacuo*.

General Procedure N: Boc and *tert*-butyl deprotection.

A pseudo-tripeptide (0.2 mmol) was dissolved in dichloromethane (5 ml) under a nitrogen atmosphere. Trifluoroacetic acid (2 ml) was added and the solution was stirred at rt for 3 h. The solvent was removed *in vacuo* and the residue dissolved in toluene, and the solution re-evaporated to give the crude.

General Procedure O1: Intramolecular lactamization.

A macrocyclic precursor was dissolved in DMF. EDCI (1.2 equiv), HOAt (1.2 equiv) and DIPEA (2.4 equiv) were added and reaction solution stirred at rt overnight. The product was extracted with ethyl acetate, washed with distilled water, and dried with brine and $MgSO_4$ and the solvent was removed *in vacuo*.

General Procedure O2: Intramolecular lactamization

To a macrocyclic precursor dissolved in DMF (0.1 M), was added HATU (1.2 equiv) and DIPEA (2.4 equiv) and the reaction mixture was stirred at rt overnight. The solution was diluted by ethyl acetate, washed by distilled water, dried with brine and $MgSO_4$ and solvent was removed *in vacuo*.

General Procedure P: Intramolecular nucleophilic substitution.

The iodide (0.01 mol), K_2CO_3 (1.5 equiv) and Cs_2CO_3 (0.1 equiv) were dissolved in dry acetonitrile (0.3 M) under a nitrogen atmosphere and reacted under reflux conditions overnight. The reaction mixture was cooled to rt and the solvent removed *in vacuo* to give a yellow residue. This residue was partitioned between ethyl acetate and aq. HCl (1M). The organic phase was separated and washed with aq. HCl (1M) and brine, dried over $MgSO_4$ and the solvent was removed *in vacuo* to give the crude product. To solid was dissolved in ethyl acetate and activated charcoal added and the mixture stirred overnight at rt. The charcoal was filtrated and the solvent was removed *in vacuo*.

General Procedure Q: *N*-Fmoc protection

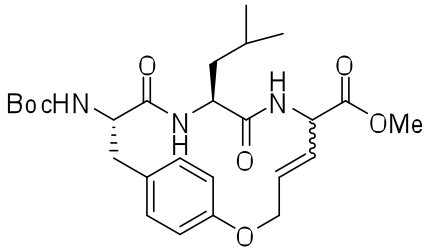
To a solution of an amino acid derivative (1 equiv) dissolved in 10% $NaCO_3$ (5 equiv) on an ice bath was added a solution of Fmoc-C1 (1 equiv) in ether. The mixture was stirred at rt for 2 h, diluted with distilled water, and extracted twice with ether to remove small amounts of 9-fluorenylmethanol and the high-melting polymer, dibenzofulvene. The aqueous layer was cooled in an ice bath and acidified with concentrated HCl to pH 2. The white precipitate was extracted with ethyl acetate and the organic layer was washed with water, dried over $MgSO_4$ and concentrated *in vacuo*.

General Procedure R: Fmoc deprotection

To a solution of *N*-Fmoc protected amino acid in acetonitrile (0.1 M) was added diethylamine (20 equiv). The reaction mixture was stirred overnight at rt and the solvent was removed *in vacuo.*

7.2: Experimental work described in chapter 2

(*E*)-(6*S*,9*S*,12*S*)-12-*tert*-Butoxycarbonylamino-9-isobutyl-8,11-dioxo-2-oxa-7,10-diazabicyclo[12.2.2]octadeca-1(17),4,14(18),15-tetraene-6-carboxylic acid methyl ester (2.1).



2.1

RCM under thermal reflux: Diene **2.5** (100 mg, 0.18 mmol) was subjected to RCM (General procedure C1) and the crude product was purified by flash chromatography on silica gel and elution with ethyl acetate/petroleum ether (3:2) to give **2.1**, a white solid (44 mg, 49%) as a mixture of as a 19(*E*):1(*Z*) mixture of isomers.

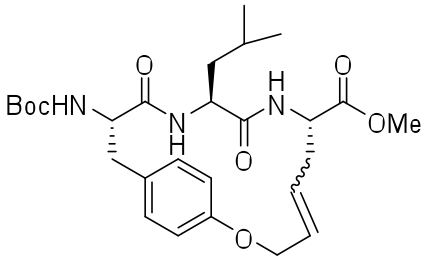
RCM under microwave irradiation: Diene **2.5** (100 mg, 0.18 mmol) was subjected to RCM (General procedure C2) and the crude product was purified by flash chromatography on silica gel and elution with ethyl acetate/petroleum ether (3:2) to give **2.1**, a white solid (52 mg, 58%) as a mixture of as a 20(*E*):1(*Z*) mixture of isomers.

RCM under thermal reflux with chlorodicyclohexylborane: Diene **2.5** (100 mg, 0.18 mmol) was subjected to RCM (General procedure C3) and the crude product was purified by flash chromatography on silica gel and elution with ethyl acetate/petroleum ether (3:2) to give **2.1**, a white solid (45 mg, 50%) as a mixture of as a 19(*E*):1(*Z*) mixture of isomers.

RCM under microwave irradiation with chlorodicyclohexylborane: Diene **2.5** (100 mg, 0.18 mmol) was subjected to RCM (General procedure C4) and the crude product was purified by flash chromatography on silica using a gradient of ethyl acetate and petroleum ether to give **2.1**, a white solid (43 mg, 47%) as a mixture of as a 20(*E*):1(*Z*) mixture of isomers. Mp 241-243 °C; ^{1}H NMR for major isomer from mixture (500 MHz, $CDCl_3$): δ 7.15-7.17 (1H, m, *H*ArO), 6.87 (2H, m, *H*ArO), 6.59-6.61(1H, m, *H*ArO), 6.31 (1H, d, *J* = 7.3 Hz, N*H*), 5.88 (1H, d, *J* = 8.5 Hz, N*H*), 5.72 (1H, ddd, *J* = 15.5, 8.0 and 4.0 Hz, OCH_2CHCH), 5.42 (1H, dd, *J* = 15.5 and 8.5 Hz, OCH_2CHCH), 5.35 (1H, d, *J* = 8.5 Hz, N*H*), 4.89 (1H, app t, *J* = 8.0 and 8.0 Hz, $CHCO_2CH_3$), 4.67 (2H, m,

OC*H*$_2$CHCH), 4.26-4.31 (1H, dd, *J* = 15.0, 8.0 Hz, C*H*CH$_2$CH(CH$_3$)$_2$), 4.15 (1H, m, C*H*CH$_2$ArO), 3.78 (3H, s, OC*H*$_3$), 3.03 (1H, dd, *J* = 12.5 and 4.5 Hz, CHC*H*HArO), 2.69 (1H, app t, *J* = 12.5 and 12.5 Hz, CHCH*H*ArO), 1.39-1.57 (12H, m, CHCH$_2$C*H*(CH$_3$)$_2$, CHC*H*$_2$CH(CH$_3$)$_2$ and (C*H*$_3$)$_3$), 0.87 (6H, m, 2 x C*H*$_3$). ^{13}C NMR (75 MHz, CDCl$_3$): δ 170.7, 170.1, 155.3, 155.1, 130.8, 130.0, 129.5, 129.4, 128.8, 119.4, 115.9, 79.6, 67.0, 57.3, 53.9, 52.8, 50.9, 42.8, 38.7, 28.3, 24.6, 22.7, 22.6. HRMS (ES) 504.2727 (MH$^+$); $C_{26}H_{38}N_3O_7$ requires 504.2710.

(*E*)/(*Z*)-(7*S*,10*S*,13*S*)-13-*tert*-Butoxycarbonylamino-10-isobutyl-9,12-dioxo-2-oxa-8,11-diaza-bicyclo[13.2.2]nonadeca-1(18),4,15(19),16-tetraene-7-carboxylic acid methyl ester (2.2).



2.2

RCM under thermal reflux: Diene **2.6** (100 mg, 0.18 mmol) was subjected to RCM (General procedure C1) and the crude product was purified by flash chromatography on silica gel and elution with ethyl acetate/petroleum ether (1:1) to give **2.2**, a white solid (21.8 mg, 22%) as a mixture of as a 10(*E*):1(*Z*) mixture of isomers.

RCM under microwave irradiation: Diene **2.6** (100 mg, 0.18 mmol) was subjected to RCM (General procedure C2) and the crude product was purified by flash chromatography on silica gel and elution with ethyl acetate/petroleum ether (1:1) to give **2.2**, a white solid (35 mg, 37%) as a mixture of as a 9(*E*):1(*Z*) mixture of isomers.

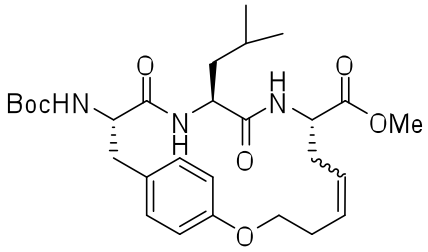
RCM under thermal reflux with chlorodicyclohexylborane: Diene **2.6** (100 mg, 0.18 mmol) was subjected to RCM (General procedure C3) and the crude product was purified

by flash chromatography on silica gel and elution with ethyl acetate/petroleum ether (1:1) to give **2.2**, a white solid (77 mg, 82%) as a mixture of as a 9(*E*):1(*Z*) mixture of isomers.

RCM under microwave irradiation with chlorodicyclohexylborane: Diene **2.6** (100 mg, 0.18 mmol) was subjected to RCM (General procedure C4) and the crude product was purified by flash chromatography on silica gel and elution with ethyl acetate/petroleum ether (1:1) to give **2.2**, a white solid (85 mg, 90%) as a mixture of as a 9(*E*):1(*Z*) mixture of isomers.

RCM under thermal reflux with titanium(IV) isopropoxide: Diene **2.6** (50 mg, 0.09 mmol) was subjected to RCM (General procedure C5) and the crude product was purified by flash chromatography on silica gel and elution with ethyl acetate/petroleum ether (1:1) to give **2.2**, a white solid (16 mg, 34%) as a mixture of as a 9(*E*):1(*Z*) mixture of isomers. ^{1}H NMR for major isomer from mixture (500 MHz in $CDCl_3$): δ 7.01 (2H, m, *H*ArO), 6.78 (2H, m, *H*ArO), 5.87 (1H, d, *J* = 8.5 Hz, N*H*), 5.78 (1H, d, *J* = 7.0 Hz, N*H*), 5.54 (1H, app dt, *J* = 15.5, 3.8 and 3.8 Hz, $OCH_2CHCHCH_2$), 5.44 (1H, ddd, *J* = 15.5, 6.5 and 1.5 Hz, $OCH_2CHCHCH_2$), 5.34 (1H, d, *J* = 8.5 Hz, N*H*), 4.73 (1H, ddd, *J* = 9.0, 8.5 and 3.0 Hz, $CHCO_2CH_3$), 4.55-4.68 (2H, m, $OCH_2CHCHCH_2$), 4.09-4.20 (2H, m, $CHCH_2ArO$ and $CHCH_2CH(CH_3)_2$), 3.74 (3H, s, OCH_3), 3.08 (1H, dd, *J* = 12.5 and 5.0 Hz, CHCHHPh), 2.65-2.74 (1H, m, CHCHHPh), 2.26-2.34 (2H, m, $CH_2CHCO_2CH_3$), 1.82-1.90 (1H, m, $CHCH_2CH(CH_3)_2$), 1.52-1.58 (2H, m, $CHCH_2CH(CH_3)_2$), 1.44 (9H, s, $(CH_3)_3$), 0.84-0.88 (6H, m, $CHCH_2CH(CH_3)_2$). ^{13}C NMR for major isomer from mixture (75 MHz, $CDCl_3$): δ 171.9, 171.0, 170.7, 156.1, 155.0, 129.7, 128.4, 128.1, 127.5, 115.8, 79.8, 66.4, 57.0, 52.5, 51.8, 51.6, 42.8, 38.8, 34.7, 28.3, 24.5, 24.3, 22.7, 22.5. HRMS (ES) 518.2869 (MH^+). $C_{27}H_{40}N_3O_7$ requires 518.2866.

(*E*)/(*Z*)-(8*S*,11*S*,14*S*)-14-*tert*-Butoxycarbonylamino-11-isobutyl-10,13-dioxo-2-oxa-9,12-diazabicyclo[14.2.2]icosa-1(19),5,16(20),17-tetraene-8-carboxylic acid methyl ester (2.3).



2.3

RCM under thermal reflux: Diene **2.7** (100 mg, 0.17 mmol) was subjected to RCM (General procedure C1) and the crude product was purified by flash chromatography on silica gel and elution with ethyl acetate/petroleum ether (1:1) to give **2.3**, a white solid (46 mg, 51%) as a mixture of as a 1.3(*E*):1(*Z*) mixture of isomers.

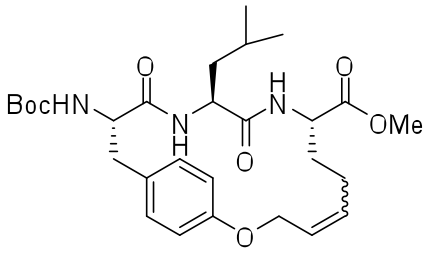
RCM under microwave irradiation: Diene **2.7** (100 mg, 0.17 mmol) was subjected to RCM (General procedure C2) and the crude product was purified by flash chromatography on silica gel and elution with ethyl acetate/petroleum ether (1:1) to give **2.3**, a white solid (52 mg, 58%) as a mixture of as a 1.7(*E*):1(*Z*) mixture of isomers.

RCM under thermal reflux with chlorodicyclohexylborane: Diene **2.7** (100 mg, 0.17 mmol) was subjected to RCM (General procedure C3) and the crude product was purified by flash chromatography on silica gel and elution with ethyl acetate/petroleum ether (1:1) to give **2.3**, a white solid (66 mg, 74%) as a mixture of as a 1.9(*E*):1(*Z*) mixture of isomers.

RCM under microwave irridation with chlorodicyclohexylborane: Diene **2.7** (100 mg, 0.17 mmol) was subjected to RCM (General procedure C4) and the crude product was purified by flash chromatography on silica gel and elution with ethyl acetate/petroleum ether (1:1) to give **2.3**, a white solid (85 mg, 96%) as a mixture of as a 1.8(*E*):1(*Z*) mixture of isomers. ^{1}H NMR of the major isomer from the mixture (500 MHz in $CDCl_3$): δ 7.09 (2H, app d, *J* = 8.0 Hz, *H*ArO), 6.75 (2H, d, *J* = 8.0 Hz, *H*ArO), 6.17 (1H, d, *J* = 7.5 Hz, N*H*), 6.12 (1H, d, *J* = 8.0 Hz, N*H*), 5.57 (1H, *m*,$OCH_2CH_2CHCHCH_2$), 5.24 (1H, d, *J* = 8.5 Hz, N*H*), 4.93 (1H, ddd, *J* = 15.0, 7.0, 5.3 Hz, $OCHHCH_2CHCHCH_2$), 4.45

(1H, ddd, *J* = 8.8, 8.6, 3.4 Hz, C*H*CO_2CH_3), 4.10-4.32 (4H, m, C*H*CH_2ArO, C*H*$CH_2CH(CH_3)_2$ and CH_2OAr), 3.74 (3H, s, OCH_3), 3.01 (1H, dd *J* = 12.0, 4.7 Hz, CHC*H*HAr), 2.82-2.84 (1H, m, CHCH*H*Ar), 2.57 (1H, m, CHCHC*H*HCHCO_2Me), 2.37-2.48 (2H, m, OCH_2CH_2CH), 2.27 (1H, m, CHCHCH*H*CHCO_2Me), 1.47-1.60 (3H, m, CHCH_2C*H*$(CH_3)_2$ and CHCH_2CH$(CH_3)_2$), 1.45 (9H, s, C(CH_3)$_3$), 0.84-0.90 (6H, m, *J* = 5.8 Hz, $CHCH_2$CH(CH_3)$_2$). ^{13}C NMR for major isomer from the mixture (75 MHz, $CDCl_3$): δ 171.9, 171.0, 170.9, 170.4, 156.6, 155.1, 130.7, 130.0, 128.9, 126.1, 115.8, 79.9, 65.5, 56.8, 52.5, 52.4, 51.6, 42.1, 38.0, 35.4, 30.6, 28.3, 24.3, 22.7, 22.4. Selected ^{1}H NMR signals for minor isomer from the mixture (500 MHz in $CDCl_3$): δ 7.12 (2H, d, *J* = 8.5 Hz, *H*ArO), 6.79 (2H, d, *J* = 8.5 Hz, *H*ArO), 6.37 (1H, d, *J*=7.5 Hz, N*H*), 6.28 (1H, d, *J* = 8.5 Hz, N*H*), 5.56 (1H, m, $OCH_2CH_2CHCHCH_2$), 5.47 (1H, dd, *J* = 10.6, 8.0 Hz, $OCHHCH_2CHCHCH_2$), 4.09-4.33 (5H, m, C*H*CH_2ArO, C*H*$CH_2CH(CH_3)_2$, CH_2OAr and C*H*CO_2CH_3, 3.71 (3H, s, OCH_3), 2.99 (1H, m, CHC*H*HAr), 2.86 (1H, m, CHCH*H*Ph), 2.49 (1H, m, C=CHC*H*HCHCO_2Me), 2.32 (1H, m, C=CHCH*H*CHCO_2Me), 1.90-2.05 (2H, m, OCH_2CH_2CH), 1.47-1.60 (3H, m, CHCH_2C*H*$(CH_3)_2$ and CHCH_2CH$(CH_3)_2$), 1.45 (9H, s, C(CH_3)$_3$), 0.84-0.90 (6H, m, $CHCH_2$CH(CH_3)$_2$). Selected ^{13}C NMR signals for minor isomer from the mixture (75 MHz, $CDCl_3$): δ 172.1, 171.0, 170.4, 170.3, 155.9, 155.3, 128.7, 128.4, 128.2, 126.6, 115.7, 80.1, 65.8, 55.9, 52.6, 52.3, 50.8, 40.8, 37.5, 31.2, 28.3, 25.8, 22.9, 22.0. HRMS (ES) 532.3034 (MH^+) $C_{28}H_{42}N_3O_7$; requires 532.3023.

(*Z*)-(8*S*,11*S*,14*S*)-14-*tert*-Butoxycarbonylamino-11-isobutyl-10,13-dioxo-2-oxa-9,12-diaza-bicyclo[14.2.2]icosa-1(19),4,16(20),17-tetraene-8-carboxylic acid methyl ester (2.4).



2.4

RCM under thermal reflux: Diene **2.8** (500 mg, 0.89 mmol) was subjected to RCM (General procedure C1) and the crude product was purified by flash chromatography on silica gel and elution with ethyl acetate/petroleum ether (3:2) to give a white solid (102 mg) as a mixture of 17- and 18-membered macrocyclic products.

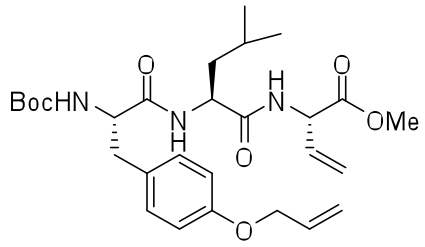
RCM under microwave irradiation: Diene **2.8** (500 mg, 0.89 mmol) was subjected to RCM (General procedure C2) and the crude product was purified by flash chromatography on silica gel and elution with ethyl acetate/petroleum ether (3:2) to give a white solid (169 mg) as a mixture of 16-, 17- and 18-membered macrocyclic products.

RCM under thermal reflux with chlorodicyclohexylborane: Diene **2.7** (500 mg, 0.89 mmol) was subjected to RCM (General procedure C3) and the crude product was purified by flash chromatography on silica gel and elution with ethyl acetate/petroleum ether (3:2) to give a white solid (240mg) as a mixture of 17- and 18-membered macrocyclic products.

RCM under microwave irradiation with chlorodicyclohexylborane: Diene **2.7** (500 mg, 0.89 mmol) was subjected to RCM (General procedure C4) and the crude product was purified by flash chromatography on silica gel and elution with ethyl acetate/petroleum ether (3:2) to give a white solid (191 mg) as a mixture of 16-, 17- and 18-membered macrocyclic products. ^{1}H NMR for major 18-membered macrocycle **2.4** from the mixture (500 MHz in $CDCl_3$): δ 7.07 (2H, app d, *J* = 8.0 Hz, *H*ArO), 7.00 (2H, app d, *J* = 8.0 Hz, *H*ArO), 6.80 (2H, app d, *J* = 8.5 Hz, *H*ArO), 6.75 (1H, app d, *J* = 8.5 Hz, *H*ArO), 6.21 (1H, d, *J* = 7.5 Hz, N*H*), 6.15 (1H, d, *J* = 8.0 Hz, N*H*), 6.06-6.10 (1H, m, N*H*), 5.84 (1H, d, *J* = 8.5 Hz, N*H*), 5.77 (1H, d, *J* = 7.5 Hz, N*H*), 5.46-5.70 (2H, m, OCH_2CHCH and OCH_2CHCH), 5.32-5.35 (1H, d, *m*, N*H*), 5.26-5.28 (1H, m, N*H*), 4.60-4.62 (2H, m, OCH_2CHCH), 4.49-56 (1H, m, $CHCO_2CH_3$), 4.34-4.44 (1H, m, $CHCH_2ArO$), 4.15-4.30 (1H, m, $CHCH_2CH(CH_3)_2$), 3.75 (3H, s, OCH_3), 3.74 (3H, s, OCH_3), 3.73 (3H, s, OCH_3), 2.85-3.05 (2H, m, $CHCH_2ArO$), 2.64-2.74 (2H, m, $CH_2CH_2CHCO_2CH_3$), 2.05-2.15 (2H, m, $CH_2CHCO_2CH_3$), 1.89-1.96 (1H, m, $CHCH_2CH(CH_3)_2$), 1.53-1.63 (2H, m, $CHCH_2CH(CH_3)_2$), 1.41-1.52 (9H, m, $(CH_3)_3$), 0.86-0.89 (6H, m, 2 x CH_3). MS (ES)

532.35 (18-membered macrocycle $[M+H]^+$, 40%); 518.35 (17-membered macrocycle $[M+H]^+$, 30%); 504.35 (16-membered macrocycle $[M+H]^+$, 10%).

(*S*)-2-{(*S*)-2-[(*S*)-3-(4-Allyloxy-phenyl)-2-*tert*-butoxycarbonylamino-propionyl amino]-4-methyl-pentanoylamino}-but-3-enoic acid methyl ester (2.5).[1]



2.5

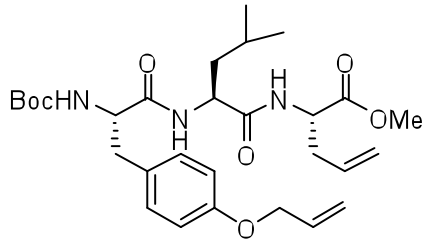
Carboxylic acid **2.12** (1.5 g, 3.45 mmol) was coupled with vinyl-Gly-OMe **2.14** (General Procedure A1). The crude product was purified by flash chromatography on silica and elution with ethyl acetate/ petroleum ether (1:5) to give **2.5** as a glassy white solid (1.39 g, 76 %). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.10 (2H, d, *J* = 8.3 Hz, *H*ArO), 6.95 (1H, d, *J* = 6.3 Hz, N*H*), 6.80 (2H, d, *J* = 8.5 Hz, *H*ArO), 6.31 (1H, d, *J* = 6.5 Hz, N*H*), 6.02-6.10 (1H, m, OCH_2CHCH_2), 5.85-5.95 (1H, m, $CHCHCH_2$), 5.25-5.40 (4H, m, OCH_2CHCH_2 and $CHCHCH_2$), 5.05 (1H, m, $CHCO_2CH_3$), 4.92 (1H, br s, N*H*), 4.42-4.51 (3H, m, $CHCH_2CH(CH_3)_2$ and OCH_2CHCH_2), 4.29 (1H, m, $CHCH_2ArO$), 3.75 (3H, s, OCH_3), 3.01 (2H, m, $CHCH_2ArO$), 1.61-1.72 (2H, m, $CHCHHCH(CH_3)_2$ and $CHCH_2CH(CH_3)_2$), 1.38 (9H, s, $(CH_3)_3$), 0.85-0.91 (6H, m, $CHCH_2CH(CH_3)_2$).

Lit cit: [1]Abell, A. D.; Jones, M. A.; Coxon, J. M.; Morton, J. D.; Aitken, S. G.; McNabb, S. B.; Lee, H. Y. Y.; Mehrtens, J. M.; Alexander, N. A.; Stuart, B. G.; Neffe, A. T.; Bickerstaffe, R. Molecular *Angew. Chem. Int. Ed.*, **2009**, *48*, 1455 –1458.

mp 102-104 ºC; ^{1}H NMR (500 MHz, $CDCl_3$): 7.08 (2H, app d, *J* = 8.5 Hz, *H*ArO), 6.96 (1H, d, *J* = 6.5 Hz, N*H*), 6.81 (2H, app d, *J* = 8.5 Hz, *H*ArO), 6.49 (2H, d, *J* = 7.0 Hz, N*H*), 6.03 (1H, m, OCH_2CHCH_2), 5.88 (1H, m, $CHCHCH_2$), 5.42-5.22 (4H, m, OCH_2CHCH_2 and $CHCHCH_2$), 5.11-5.02 (2H, m, $CHCO_2CH_3$ and N*H*), 4.61-4.40 (3H, m, $CHCH_2CH(CH_3)_2$ and OCH_2CHCH_2), 4.32 (1H, m, $CHCH_2ArO$), 3.76 (3H, s, OCH_3), 2.97 (2H, m, $CHCH_2ArO$), 1.66 (1H, m, $CHCHHCH(CH_3)_2$), 1.57 (1H, m, $CHCH_2CH(CH_3)_2$), 1.48 (1H, m, $CHCHHCH(CH_3)_2$), 1.39 (9H, s $C(CH_3)_3$), 0.90 (6H, m, 2 x CH_3); ^{13}C NMR (75 MHz,

$CDCl_3$): 171.7, 171.4, 170.5, 157.5, 155.7, 133.2, 131.6, 130.3, 130.2, 128.6, 117.9, 117.5, 114.7, 80.0, 68.8, 55.7, 55.6, 54.4, 52.6, 51.6, 40.9, 37.1, 28.2, 24.5, 22.7, 22.1; HRMS (ES) 532.3027 (MH_+); $C_{28}H_{42}N_3O_7$ requires 532.3023.

(*S*)-2-{(*S*)-2-[(*S*)-3-(4-Allyloxyphenyl)-2-*tert*-butoxycarbonylaminopropionylamino]-4-methylpentanoylam-ino}-pent-4-enoic acid methyl ester (2.6).[1]



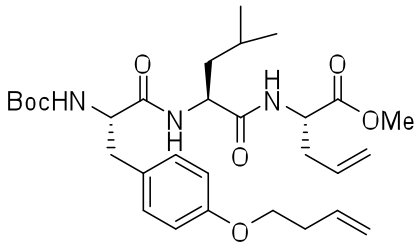
2.6

Carboxylic acid **2.12** (4.35 g, 10 mmol) was coupled with allyl-Gly-OMe **2.13** (General Procedure A1) and the crude material was purified by flash chromatography on silica gel and elution with ethyl acetate/petroleum ether (2:3) to give.**2.6** as a white solid (4.17 g, 77%). ^{1}H NMR (500 MHz in $CDCl_3$) δ 7.09 (2H, m, *H*ArO), 6.87 (1H, d, *J* = 7.7 Hz, N*H*), 6.82 (2H, d, *J* = 8.5 Hz, *H*ArO), 6.67 (1H, d, *J* = 7.6Hz, N*H*), 6.04-6.06 (1H, tdd, *J* = 17.0, 10.5, 5.2 and 5.2 Hz, OCH_2CHCH_2), 5.74-5.79 (1H, m, $CHCH_2CHCH_2$), 5.38 (1H, dd, *J* = 17.5 and 1.5 Hz, OCH2CHCH_{trans}H), 5.27 (1H, dd, *J* = 10.5 and 1.5 Hz, OCH_2CHCHH_{cis}), 5.05-5.09 (2H, m, OCH2CHCH_2), 4.99 (1H, d, *J* = 7.5Hz, N*H*), 4.52-4.54 (1H, m, C*H*$CH_2CH(CH_3)_2$), 4.49 (2H, m, OC$H_2$$CHCH_2$), 4.43-4.48 (1H, m, C*H*CH_2CHCH_2), 4.33 (1H, m, C*H*CH_2Ph), 3.73 (3H, s, CO_2CH_3), 2.94-3.05 (2H, m, CHCH_2Ph), 2.04-2.09 (2H, m, CHC$H_2$$CHCH_2$), 1.91-1.94 (1H, m, CHC*H*$HCH(CH_3)_2$), 1.74-1.78 (1H, m, CHCH*H*$CH(CH_3)_2$), 1.43-1.50 (1H, m, $CHCH_2CH(CH_3)_2$) 1.39 (9H, s, $C(CH_3)_3$), 0.91 (6H, m, $CHCH_2CH(CH_3)_2$). ^{13}C NMR (75 MHz in $CDCl_3$). δ 172.7, 171.8, 171.3, 157.8, 155.4, 137.0, 133.5, 132.0, 130.5, 130.1, 128.8, 119.2, 119.0, 117.9, 115.1, 80.6, 69.0, 56.0, 52.5, 52.0, 41.1, 40.7, 37.2, 36.2, 28.2, 24.4, 22.8, 22.2. MS (ES) 546.31 (MH^+) $C_{29}H_{43}N_3O_7$ requires 546.31.

Lit cit: [1]Abell, A. D.; Jones, M. A.; Coxon, J. M.; Morton, J. D.; Aitken, S. G.; McNabb, S. B.; Lee, H. Y. Y.; Mehrtens, J. M.; Alexander, N. A.; Stuart, B. G.; Neffe, A. T.; Bickerstaffe, R. Molecular *Angew. Chem. Int. Ed.*, **2009**, *48*, 1455 –1458.

mp 105-110 ºC; ^{1}H-NMR (500 MHz, $CDCl_3$): 7.09 (2H, app d, *J* = 8.5 Hz, *H*ArO), 6.82 (2H, app d, *J* = 8.5 Hz, *H*ArO), 6.65 (1H, d, *J* = 7.5 Hz, N*H*), 6.47 (1H, d, *J* = 8.0 Hz, N*H*), 6.04 (1H, app ddt, *J* = 17.0, 10.5, 5.25 and 5.25 Hz, OCH_2CHCH_2), 5.62-5.71 (1H, m, $CHCH_2CHCH_2$), 5.39 (1H, dd, *J* = 17.5 and 1.5 Hz, $OCH_2CHCH_{trans}H$), 5.26 (1H, dd, *J* = 10.5 and 1.5 Hz, $OCH_2CHCH_{cis}H$), 5.03-5.12 (2H, m, OCH_2CHCH_2), 4.99 (1H, d, *J* = 7.5 Hz, N*H*), 4.58 (1H, dd, *J* = 13.5 and 6.5 Hz, $CHCH_2CH(CH_3)_2$), 4.49 (2H, app d, *J* = 5.0 Hz, OCH_2CHCH_2), 4.40-4.45 (1H, m, $CHCO_2CH_3$), 4.25-4.34 (1H, m, $CHCH_2ArO$), 3.73 (3H, s, OCH_3), 2.96-3.05 (2H, m, $CHCH_2ArO$), 2.45-2.61 (2H, m, $CHCH_2CHCH_2$), 1.52-1.68 (2H, m, $CHCHCH_2(CH_3)_2$), 1.43-1.50 (1H, m, $CHCHCH_2(CH_3)_2$) 1.39 (9H, s, $C(CH_3)_3$), 0.90 (3H, d, *J* = 6.5 Hz, CH_3), 0.89 (3H, d, *J* = 6.5 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): _ 171.8, 171.5, 171.3, 157.5, 155.4, 133.2, 132.2, 132.0, 130.3, 130.1, 128.5, 119.2, 119.0, 117.6, 114.8, 80.2, 68.7, 55.6, 52.3, 51.7, 40.9, 40.7, 37.0, 36.2, 28.2, 24.4, 22.8, 22.0; HRMS (ES) 546.3180 (MH^+); $C_{29}H_{43}N_3O_7$ requires 546.3179.

(*S*)-2-{(*S*)-2-[(*S*)-3-(4-But-3-enyloxyphenyl)-2-tertbutoxycarbonyl aminopropionyl amino]-4-methylpentan-oylamino}-pent-4-enoic acid methyl ester (2.7).[1]



2.7

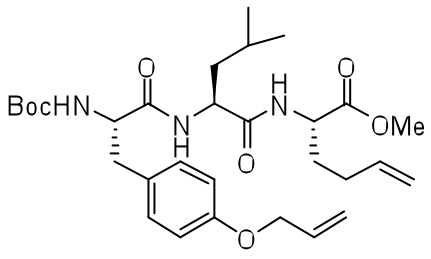
Carboxylic acid **2.19** (1.2 g, 2.6 mmol) was coupled with allyl-Gly-OMe (General Procedure A1). The crude product was purified by flash chromatography on silica gel and elution with ethyl acetate/petroleum ether (2:3) to give **2.7** as a white solid (1.23 g, 82%). Mp 86-88 ºC. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.07 (2H, d, *J* = 8.6 Hz, *H*ArO), 6.79 (2H, app d, *J* = 8.5 Hz, *H*ArO), 6.71 (1H, d, *J* = 7.7 Hz, N*H*), 6.52 (1H, d, *J* = 8.0 Hz, N*H*), 5.85-5.88 (1H, dddd, *J* = 17.0, 10.0, 7.5 and 3.7 Hz, $OCH_2CH_2CHCH_2$), 5.85-5.88 (1H, m, $CHCH_2CHCH_2$), 5.03-5.16 (4H, m, $OCH_2CH_2CHCH_2$ and $CHCH_2CHCH_2$), 4.56-4.60

(1H, m, C*H*CO_2CH_3), 4.50 (1H, m, C*H*$CH_2CH(CH_3)_2$), 4.31 (1H, m, C*H*CH_2ArO), 3.96 (2H, t, *J* = 6.6 and 6.8 Hz,O$CH_2CH_2CHCH_2$), 3.72 (3H, s, OCH_3), 2.98 (2H, m, CHCH_2ArO), 2.45-2.56 (4H, m, CHCH_2CHCH_2 and OCH_2CH_2CHCH_2, 1.54-1.66 (2H, m, CHCH_2CH$(CH_3)_2$), 1.43-1.48 (1H, m, CHCH_2C*H*$(CH_3)_2$), 1.38 (9H, s, C$(CH_3)_3$), 0.89 (6H, m, CHCH_2CH$(CH_3)_2$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.0, 171.7, 171.5, 158.2, 155.4, 134.4, 132.3, 130.5, 130.2, 128.6, 118.9, 116.9, 114.5, 79.8, 67.0, 55.6, 52.2, 51.8, 51.5, 41.2, 37.2, 36.5, 33.8, 28.5, 24.7, 23.1, 22.3. MS (ES) 560.33 (MH^+); $C_{30}H_{45}N_3O_7$ requires 560.33.

Lit cit: [1]Abell, A. D.; Jones, M. A.; Coxon, J. M.; Morton, J. D.; Aitken, S. G.; McNabb, S. B.; Lee, H. Y. Y.; Mehrtens, J. M.; Alexander, N. A.; Stuart, B. G.; Neffe, A. T.; Bickerstaffe, R. Molecular *Angew. Chem. Int. Ed.*, **2009**, *48*, 1455 –1458.

mp 88-90 ºC; ^{1}H NMR (500 MHz, $CDCl_3$): 7.09 (2H, app d, *J* = 8.5 Hz, *H*ArO), 6.93 (1H, d, *J* = 7.5 Hz, N*H*), 6.80 (2H, app d, *J* = 8.5 Hz, *H*ArO), 6.71 (1H, d, *J* = 7.3 Hz, N*H*), 5.86 (1H, dddd, *J* = 17.0, 10.0, 7.5 and 3.5 Hz, $OCH_2CH_2CHCH_2$), 5.61-5.70 (1H, m, $CHCH_2CHCH_2$), 5.09-5.22 (4H, m, $OCH_2CH_2CHCH_2$ and $CHCH_2CHCH_2$), 4.54-4.60 (1H, m, C*H*CO_2CH_3), 4.50 (1H, dd, *J* = 13.5 and 6.5 Hz, C*H*$CH_2CH(CH_3)_2$), 4.36-4.38 (1H, dd, *J* = 8.5 and 6.5 Hz, C*H*CH_2ArO), 3.96 (2H, app t, *J* = 6.75 and 6.75 Hz, O$CH_2CH_2CHCH_2$), 3.73 (3H, s, OCH_3), 2.91 (2H, m, CHCH_2ArO), 2.45-2.58 (4H, m, CHCH_2CHCH_2 and OCH_2CH_2CHCH_2, 1.54-1.66 (2H, m, CHCH_2CH$(CH_3)_2$), 1.43-1.48 (1H, m, $CHCH_2$C*H*$(CH_3)_2$), 1.39 (9H, s, C$(CH_3)_3$), 0.86 (3H, d, *J* = 6.5 Hz, CH_3), 0.86 (3H, d, *J* = 6.5 Hz, CH_3); ^{13}C NMR (75 MHz,$CDCl_3$): 171.8, 171.6, 171.5, 157.8, 155.4, 134.4, 132.2, 130.3, 130.2, 128.6, 118.9, 116.9, 114.5, 79.8, 67.0, 55.6, 52.2, 51.8, 51.5, 41.1, 37.2, 36.3, 36.2, 33.6, 28.2, 24.4, 22.8, 22.2; HRMS (ES) 560.3346 (MH^+); $C_{30}H_{45}N_3O_7$ requires 560.3336.

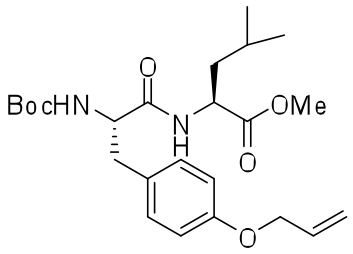
(*S*)-2-{(*S*)-2-[(*S*)-3-(4-Allyloxy-phenyl)-2-*tert*-butoxycarbonylamino-propionyl amino]-4-methyl-pentanoylamino}-hex-5-enoic acid methyl ester (2.8)



2.8

Carboxylic acid **2.12** (5.83 g, 13.4 mmol) was coupled homoallyl-Gly-OMe **2.22** (General procedure A1). The crude product was purified by flash chromatography on silica using a gradient of ethyl acetate/petroleum ether (1:1) gave **2.8** as a white solid (5.75g, 77%). Mp 125-127 ºC. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.05 (2H, app d, *J* = 8.6 Hz, *H*ArO), 6.82 (1H, br s, N*H*), 6.76 (2H, app d, *J* = 8.6 Hz, *H*ArO), 6.03 (1H, dddd, *J* = 17.3, 10.4, 5.2 and 5.2 Hz, OCH_2CHCH_2), 5.73 (1H, dddd, *J* = 17.0, 10.3, 6.6 and 6.6 Hz, $C_\alpha HCH_2CH_2CHCH_2$), 5.38 (1H, dd, *J* = 17.3, 1.5, Hz, $OCH_2CHCH_{trans}H$), 5.30 (1H, d, *J* = 7.9Hz, N*H*), 5.23 (1H, dd, J = 10.4, 1.3 Hz, OCH_2CHCHH_{cis}), 4.99 (1H, dd, *J* = 17.0, 1.6, Hz $C_\alpha HCH_2CH_2CHCH_{trans}H$), 4.95 (1H, dd, *J* = 10.3, 1.0 Hz $C_\alpha HCH_2CH_2CHCHH_{cis}$), 4.52 (2H, m, $CHCO_2CH_3$ and $CHCH_2CH(CH_3)_2$), 4.45 (2H, d, *J* = 5.2 Hz, OCH_2CHCH_2), 4.38 (1H, br s, $CHCH_2ArO$), 3.68 (3H, s, OCH_3), 2.92 (2H, m, $CHCH_2ArO$), 2.04 (2H, m, $C_\alpha HCH_2CH_2CHCH_2$), 1.87 (1H, m, $CHCH_2CH_2CHCH_2$), 1.73 (1H, m, CHCHHCH2CHCH2), 1.61 (1H, m, $CHCHHCH(CH_3)_2$), 1.56 (1H, m, $CHCH_2CH(CH_3)_2$), 1.45 (1H, m, $CHCHHCH(CH_3)_2$), 1.33 (9H, s, $C(CH_3)_3$), 0.85 (6H, m, 2 x CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.7, 171.9, 171.8, 157.7, 155.8, 136.9, 133.5, 130.5, 130.4, 128.9, 117.7, 116.1, 114.9, 80.2, 68.9, 55.9, 52.4, 51.9, 51.8, 41.3, 37.3, 35.3, 31.4, 29.7, 28.4, 24.7, 23.0, 22.9, 22.4, 18.2. HRMS (ES) 560.3326 (MH^+); $C_{30}H_{46}N_3O_7$ requires 560.3336.

(*S*)-2-[(*S*)-3-(4-Allyloxy-phenyl)-2-*tert*-butoxycarbonylamino-propionylamino]-4-methyl-pentanoic acid methyl ester (2.11).[1]



2.11

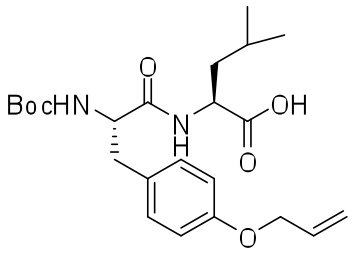
N-Boc-Tyr(O-allyl)-H **2.9** (17 g, 55 mmol) was coupled with Leu-OMe·HCl **2.10** (General Procedure A1). The crude product was purified by flash chromatography on silica using a gradient of ethyl acetate and petroleum ether to give as a glassy white solid

2.11 (19.5 g, 79 %). Mp 78-80 ºC. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.10 (2H, m, *H*ArO), 6.82 (2H, m, *H*ArO), 6.30 (1H, d, *J* = 7.9 Hz, N*H*), 6.01 (1H, m, OCH_2CHCH_2), 5.41 (1H, dd, *J* = 14.2 and 1.5 Hz, $OCH_2CHCH_{trans}H$), 5.27 (1H, dd, *J* = 10.3 and 1.2 Hz, $OCH_2CHCH_{cis}H$), 5.09 (1H, app d, N*H*), 4.54-4.56 (1H, m, $CHCH_2CH(CH_3)_2$), 4.49 (2H, m, OCH_2CHCH_2), 4.30 (1H, m, $CHCH_2ArO$), 3.68 (3H, s, OCH_3), 2.98-3.01 (2H, m, $CHCH_2ArO$), 1.54-1.57 (2H, m, $CHCH_2CH(CH_3)_2$), 1.44-1.48 (1H, m, $CHCH_2CH(CH_3)_2$) 1.41 (9H, s, $(CH_3)_3$), 0.87 (3H, d, *J* = 6.5 Hz, CH_3), 0.86 (3H, d, *J* = 6.5 Hz, CH_3). MS (ES) 449.2655 (MH^+); $C_{24}H_{37}N_2O_6$ requires 449.2651.

Lit cit: [1]Abell, A. D.; Jones, M. A.; Coxon, J. M.; Morton, J. D.; Aitken, S. G.; McNabb, S. B.; Lee, H. Y. Y.; Mehrtens, J. M.; Alexander, N. A.; Stuart, B. G.; Neffe, A. T.; Bickerstaffe, R. Molecular *Angew. Chem. Int. Ed.*, **2009**, *48*, 1455 –1458.

^{1}H NMR (500 MHz, $CDCl_3$): 7.08 (2H, m, *H*ArO), 6.79 (2H, m, *H*ArO), 6.43 (1H, d, *J* = 7.5 Hz, N*H*), 6.01 (1H, app ddt, *J* = 17.5, 10.5, 5.75 and 5.75 Hz, OCH_2CHCH_2), 5.37 (1H, dd, *J* = 17.0 and 1.5 Hz, $OCH_2CHCH_{trans}H$), 5.24 (1H, dd, *J* = 10.5 and 1.5 Hz, $OCH_2CHCH_{cis}H$), 5.09 (1H, d, *J* = 7.0 Hz, N*H*), 4.54-4.57 (1H, m, $CHCH_2CH(CH_3)_2$), 4.47 (2H, app d, *J* = 5.5 Hz, OCH_2CHCH_2), 4.30-4.36 (1H, m, $CHCH_2ArO$), 3.66 (3H, s, OCH_3), 2.88-3.02 (2H, app d, *J* = 7.0 Hz, $CHCH_2ArO$), 1.51-1.59 (2H, m, $CHCH_2CH(CH_3)_2$), 1.41-1.48 (1H, m, $CHCH_2CH(CH_3)_2$) 1.38 (9H, s, $(CH_3)_3$), 0.87 (3H, d, *J* = 6.5 Hz, CH_3), 0.86 (3H, d, *J* = 6.5 Hz, CH_3); ^{13}C NMR (75 MHz $CDCl_3$): 174.1, 172.8, 171.4, 157.5, 155.5, 133.2, 130.4, 130.3, 128.7, 117.4, 114.7, 80.0, 68.7, 54.3, 52.1, 50.7, 41.3, 37.3, 28.2, 24.6, 22.7, 21.8; HRMS (ES) 449.2662 (MH^+); $C_{24}H_{37}N_2O_6$ requires 449.2651.

(*S*)-2-[(*S*)-3-(4-Allyloxy-phenyl)-2-*tert*-butoxycarbonylamino-propionylamino]-4-methyl-pentanoic acid (2.12).[1]



2.12

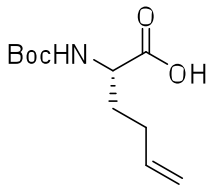
Methyl ester **2.11** (19 g, 42.4 mmol) was hydrolyzed (General procedure B) to give **2.12** as a white solid (18.5 g, 98%). ^{1}H NMR (500 MHz in $CDCl_3$): 7.11 (2H, d, *J* = 7.0 Hz,

*H*ArO), 6.82 (2H, d, *J* = 7.0 Hz, *H*ArO), 6.99 (1H, app d, N*H*), 6.03 (1H, m, OCH_2CHCH_2), 5.28-5.37 (2H, m, OCH_2CHCH_2), 5.23 (1H, br s, N*H*), 4.54-4.56 (1H, m, $CHCH_2CH(CH_3)_2$), 4.42-4.49 (2H, m, OCH_2CHCH_2 and $CHCH_2ArO$), 2.95-3.01 (2H, m, $CHCH_2ArO$), 1.53-1.68 (2H, m, $CHCHCH_2(CH_3)_2$), 1.52-1.56 (1H, m, $CHCHCH_2(CH_3)_2$), 1.39 (9H, s, $C(CH_3)_3$), 0.85-0.93 (6H, m, 2 x CH_3). ^{13}C NMR (75 MHz CDCl3): 176.1, 172.0, 157.8, 155.2, 133.5, 130.6, 128.8, 117.8, 115.1, 80.2, 69.0, 55.9, 51.1, 41.4, 37.3, 28.4, 24.9, 23.0, 20.1.

Lit cit: [1]Abell, A. D.; Jones, M. A.; Coxon, J. M.; Morton, J. D.; Aitken, S. G.; McNabb, S. B.; Lee, H. Y. Y.; Mehrtens, J. M.; Alexander, N. A.; Stuart, B. G.; Neffe, A. T.; Bickerstaffe, R. Molecular *Angew. Chem. Int. Ed.*, **2009**, *48*, 1455 –1458.

mp 72-75 ºC; ^{1}H-NMR (500 MHz in $CDCl_3$): 7.10 (2H, app d, *J* = 8.5 Hz, *H*ArO), 6.82 (2H, app d, *J* = 8.5 Hz, *H*ArO), 6.58 (1H, d, *J* = 8.0 Hz, N*H*), 6.03 (1H, app ddt, *J* = 16.0, 10.5, 5.75 and 5.75 Hz, OCH_2CHCH_2), 5.39 (1H, dd, *J* = 17.0 and 1.5 Hz, $OCH_2CHCH_{trans}H$), 5.26 (1H, dd, *J* = 10.5 and 1.5 Hz, $OCH_2CHCH_{cis}H$), 5.22 (1H, br s, N*H*), 4.54-4.58 (1H, m, $CHCH_2CH(CH_3)_2$), 4.48 (2H, app d, *J* = 5.5 Hz, OCH_2CHCH_2), 4.34-4.40 (1H, m, $CHCH_2ArO$), 2.96-3.02 (2H, m, $CHCH_2ArO$), 1.58-1.70 (2H, m, $CHCHCH_2(CH_3)_2$), 1.50-1.56 (1H, m, $CHCHCH_2(CH_3)_2$), 1.39 (9H, s, $C(CH_3)_3$), 0.91 (3H, d, *J* = 6.0 Hz, CH_3), 0.91 (3H, d, *J* = 6.0 Hz, CH_3); ^{13}C NMR (75 MHz $CDCl_3$): _ 176.1, 172.1, 157.8, 155.2, 133.6, 130.7, 128.9, 117.9, 115.1, 80.3, 69.0, 55.9, 51.1, 41.5, 37.3, 28.5, 25.0, 23.1, 20.1; HRMS (ES) 435.2515 (MH^+); $C_{23}H_{35}N_2O_6$ requires 435.2495.

(*S*)-2-*tert*-Butoxy carbonylamino -hex-5-enoic acid (2.21).

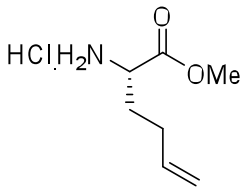


2.21

To a suspension of *N*-Boc-Lys-OH **2.20** (31.8 g, 129.3 mmol) was treated with sodium nitroprusside (58.8 g, 197.3 mmol) (General Procedure I1) and the crude product was purified by flash chromatography on silica gel and elution with ethyl acetate/petroleum ether (1:2) to give **2.21** as yellow oil (9.8 g, 30%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.75-5.81 (1H, m, $CHCH_2CH_2CHCH_2$), 5.01-5.10 (2H, m, $CHCH_2CH_2CHCH_2$), 4.02 (1H, m,

C*H*$CH_2CH_2CHCH_2$), 2.15-2.18 (2H, m, $CHCH_2CH_2CHCH_2$), 1.65-1.80 (2H, m, $CHCH_2CH_2CHCH_2$), 1.39 (9H, m, $(CH_3)_3$). ^{13}C NMR ($(CD_3)_2SO$, 75 MHz): δ 174.8, 156.2, 138.2, 116.1, 78.6, 53.5, 30.7, 30.4, 28.9, 28.6. LRMS (ES) 230.13 (MH^+); $C_{11}H_{20}NO_4$ requires 230.13.

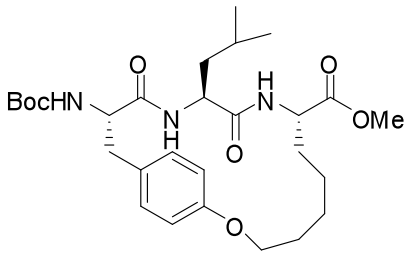
(*S*)-2-Amino-hex-5-enoic acid methyl ester (2.22)



2.22

Carboxylic acid **2.21** (2.23 g, 9.7 mmol) was treated with thionyl chloride in methanol (General Procedure E2) to give **2.22** as a yellow solid (1.85 g, 95%). ^{1}H NMR ($(CD_3)_2SO$, 300 MHz): δ 5.77 (1H, m, $CHCH_2CH_2CHCH_2$), 5.02-5.06 (2H, m, $CHCH_2CH_2CHCH_2$), 3.92 (1H, m, $CHCH_2CH_2CHCH_2$), 3.71 (1H, s, OCH_3), 2.10-2.15 (2H, m, $CHCH_2CH_2CHCH_2$), 1.82-1.89 (2H, m, $CHCH_2CH_2CHCH_2$). ^{13}C NMR ($(CD_3)_2SO$, 75 MHz): δ 170.4, 136.9, 117.2, 56.6, 52.7, 39.8, 29.6.

(8*S*,11*S*,14*S*)-14-*tert*-Butoxycarbonyl amino-11-isobutyl-10,13-dioxo-2-oxa -9,12-diaza - bicyclo[14.2.2]icosa-1(19),16(20),17-triene-8-carboxylic acid methyl ester (2.28).



2.28

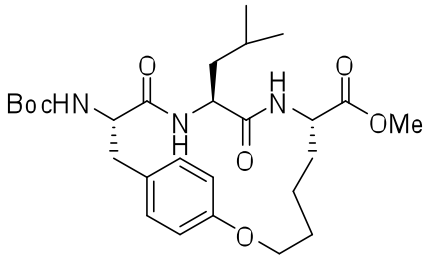
Alkene **2.4** (76 mg) prepared by RCM under thermal reflux condition (general procedure C1) was subjected to hydrogenation (General procedure D) to give a mixture of 17- and 18-membered macrocyclic products (30mg).

Alkene **2.4** (196 mg) prepared by RCM under thermal reflux with chlorodicyclohexyl borane (General procedure C2) was subjected to hydrogenation (General procedure D) to give a mixture of 16-, 17- and 18-membered macrocyclic products (147mg).

Alkene **2.4** (153 mg) prepared by RCM under microwave (General procedure C3) was subjected to hydrogenation (General procedure D) to give a mixture of 17- and 18-membered macrocyclic products (105 mg).

Alkene **2.4** (180 mg) prepared by RCM under microwave irradiation with chlorodicyclohexyl borane (General procedure C4) was subjected to hydrogenation (General procedure D) to give a mixture of 16-, 17- and 18-membered macrocyclic products (95mg). ^{1}H NMR of the major 18-memered macrocycle **2.28** (500 MHz, $CDCl_3$): δ 7.06 (2H, d, *J* = 8.2 Hz, *H*ArO), 6.75 (2H, d, *J* = 8.5 Hz, *H*ArO), 6.02 (1H, d, *J* = 7.1 Hz, N*H*), 5.84 (1H d, *J* = 8.0 Hz, N*H*), 5.20 (1H d, *J* = 8.8 Hz, N*H*), 4.37 (1H, m, C*H*CO_2CH_3), 4.15 (C*H*$CH_2CH(CH_3)_2$), 4.09-4.11 (2H, m, ArOCH*H*CH_2CH_2 and C*H*CH_2ArO), 4.01 (1H, m, ArOC*H*HCH_2CH_2), 3.71 (3H, s, OCH_3), 3.04 (1H, dd, *J* = 12.8 and 4.9 Hz, CHC*H*HArO), 2.72 (1H, t, *J* = 12.5 and 12.0 Hz, CHCH*H*ArO), 1.63-1.74 (3H, m, Ar$OCH_2CH_2CH_2$ and C*H*$HCHCO_2CH_3$), 1.51-1.60 (3H, m, CHC*H*$_2CH(CH_3)_2$ and $CHCH_2CH(CH_3)_2$), 1.43 (9H, s, $C(CH_3)_3$), 1.21-1.37 (5H, m, $ArOCH_2CH_2CH_2$, $CH_2CH_2CHCO_2CH_3$ and CH*H*$CHCO_2CH_3$), 0.86 (6H, m, 2 x CH_3). MS (ES) 534.30 (18-membered macrocycle $[M+H]^+$); 520.30 (17-membered macrocycle $[M+H]^+$); 506.28 (16-membered macrocycle $[M+H]^+$).

(7*S*,10*S*,13*S*)-13-*tert*-Butoxycarbonylamino-10-isobutyl-9,12-dioxo-2-oxa-8,11-diaza-bicyclo[13.2.2]-nonadeca-1(18),15(19),16-triene-7-carboxylic acid methyl ester (2.29).[1]



2.29

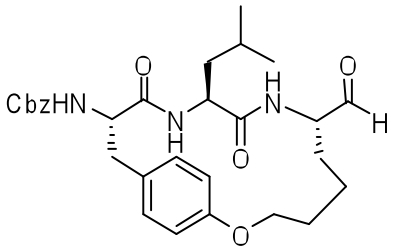
Alkene **2.2** (3.65 g, 7.1 mmol) was hydrogenated (General Procedure D) and the crude product was purified by flash chromatography on silica gel and elution with a gradient of ethyl acetate and petroleum ether to give **2.29** as a white solid (3.48 g, 95%).[1]H NMR (500 MHz in $CDCl_3$): δ 7.06 (2H, m, *H*ArO), 6.79 (2H, m, *H*ArO), 6.05 (1H, d, *J* = 7.5 Hz, N*H*), 5.78 (1H, d, *J* = 8.5 Hz, N*H*), 5.21 (1H, d, *J* = 8.5 Hz, N*H*), 4.55 (1H, m, C*H*CO_2CH_3), 4.27 (1H, m, ArOC*H*HCH_2), 4.20 (1H, m, C*H*CH_2ArO), 4.12 (1H, m, ArOCH*H*CH_2), 3.98 (1H, m, C*H*$CH_2CH(CH_3)_2$), 3.76 (3H, s, OCH_3), 3.08-3.10 (1H, m, CHCH*H*ArO), 2.60-2.64 (1H, m, CHCH*H*ArO), 1.85-1.93 (1H, m, ArO$CH_2CH_2CH_2$C*H*H), 1.65-1.70 (2H, m, ArOCH_2C_2), 1.52-1.60 (4H, m, ArO$CH_2CH_2CH_2$CH*H*, $CHCH_2$C*H*$(CH_3)_2$ and $CHCH_2CH(CH_3)_2$), 1.43 (9H, s, $(CH_3)_3$), 1.25-1.36 (2H, m, ArO$CH_2CH_2CH_2$), 0.84-1.90 (6H, m, 2 xCH_3).

Lit cit: [1]Abell, A. D.; Jones, M. A.; Coxon, J. M.; Morton, J. D.; Aitken, S. G.; McNabb, S. B.; Lee, H. Y. Y.; Mehrtens, J. M.; Alexander, N. A.; Stuart, B. G.; Neffe, A. T.; Bickerstaffe, R. Molecular *Angew. Chem. Int. Ed.*, **2009**, *48*, 1455 –1458.

mp 235-236 ºC; [1]H-NMR (500 MHz, $CDCl_3$): 7.05 (2H, m, *H*ArO), 6.79 (2H, m, *H*ArO), 6.08 (1H, d, *J* = 6.0 Hz, N*H*), 5.74 (1H, d, *J* = 8.5 Hz, N*H*), 5.22 (1H, d, *J* = 8.5 Hz, N*H*), 4.38-4.42 (1H, ddd, *J* = 8.5, 8.5 and 4.0 Hz, C*H*CO_2CH_3), 4.25-4.30 (1H, m, ArOC*H*HCH_2), 4.17-4.22 (1H, m, C*H*CH_2ArO), 4.10-4.14 (1H, m, ArOCH*H*CH_2), 3.96-4.06 (1H, dd, *J* = 13.5 and 6.5 Hz, C*H*$CH_2CH(CH_3)_2$), 3.73 (3H, s, OCH_3), 3.11 (1H, dd, *J* = 12.5 and 5.5 Hz, CHCH*H*ArO), 2.65 (1H, app t, *J* = 12.0 and 12.0 Hz CHCH*H*ArO), 1.68-1.74 (2H, m, ArOCH_2C_2), 1.49-1.63 (5H, m, $CHC_2CH(CH_3)_2$, $CHCH_2$C*H*$(CH_3)_2$ and $CH_2C_2CHCO_2CH_3$), 1.45 (9H, s, C(C*H*$_3)_3$), 1.20-1.40 (2H, m, C*H*$_2$CHCO_2CH_3), 0.82-0.85 (6H, m, 2 x C*H*$_3$); [13]C NMR (75 MHz, $CDCl_3$): 172.6, 170.8, 170.1, 157.0, 155.6, 155.1, 130.3, 130.1, 128.4, 115.9, 115.7, 79.7, 66.7, 56.8, 52.5, 51.6, 51.22, 43.1, 38.7, 31.5, 28.2, 28.2, 24.4, 22.7, 22.5, 21.4; HRMS (ES) 520.3031 (MH^+); $C_{27}H_{42}N_3O_7$ requires 520.3023.

7.3: Experimental work described in chapter 3

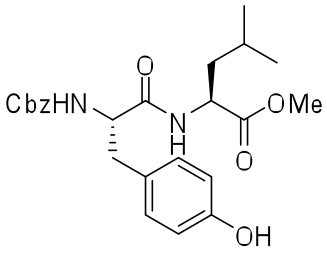
((7*S*,10*S*,13*S*)-7-Formyl-10-isobutyl-9,12-dioxo-2-oxa-8,11-diaza-bicyclo [13.2.2] nonadeca-1(18),15(19),16-trien-13-yl)-carbamic acid benzyl ester (CAT811).



CAT811

The macrocyclic alcohol **3.12** (2.0 g, 3.80 mmol) was oxidised (General procedure H1) and the crude product was recrystallised from ethyl acetate to give **CAT811** as a white solid (1.49 g, 75 %). Mp 236-238 ºC. ^{1}H NMR (500 MHz in $(CD_3)_2SO$): δ 9.33 (1H, s, C*H*O), 8.05 (1H, d, *J* = 8.0 Hz, N*H*), 7.55 (1H, d, *J* = 7.0 Hz, N*H*), 7.30-7.37 (5H, m, Ph), 7.16 (1H, m, N*H*), 7.02 (2H, m, *H*ArO), 6.77 (2H, d, *J* = 7.5 Hz, *H*ArO), 5.04 (1H, d, *J* = 12.5 Hz, PhC*H*H), 5.00 (1H, d, *J* = 12.5 Hz, PhCH*H*), 4.31-4.36 (3H, m, C*H*CH_2ArO and ArOCH_2), 4.18-4.25 (1H, m, C*H*$CH_2CH(CH_3)_2$), 4.00-4.07 (2H, m, C*H*CHO and C*H*HCHCHO), 2.86 (1H, dd, *J* = 12.0 and 5.0 Hz, CHC*H*HArO), 2.63 (1H, app t, *J* = 12.5 and 11.5 Hz, CHCH*H*ArO), 1.70-1.77 (2H, m, CH*H*CHCHO), 1.46-1.52 (1H, m, $CHCH_2CH(CH_3)_2$), 1.22-1.39 (6H, m, $CHCH_2CH(CH_3)_2$, $ArOCH_2CH_2$ and CH_2CH_2CHCHO), 0.80-0.83 (6H, m, 2 x CH_3). ^{13}C NMR (75 MHz $(CD_3)_2SO$): δ 201.8, 172.1, 170.3, 156.6, 156.1, 137.9, 130.1, 129.0, 128.4, 116.2, 66.8, 65.9 57.2, 56.8, 51.4, 44.1, 31.0, 27.6, 27.1, 24.6, 23.6, 22.2. HRMS (ES) 524.2762 (MH^+); $C_{29}H_{37}N_3O_6$ requires 524.2760.

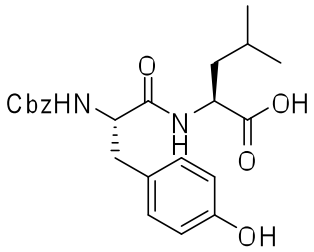
(*S*)-2-[(*S*)-2-Benzyloxycarbonylamino-3-(4-hydroxy-phenyl)-propionylamino]-4-methyl-pentanoic acid methyl ester (3.4).



3.4

Cbz-*L*-Tyrosine **3.2** (3.50 g, 11.1 mmol) was coupled with *L*-Leucine methyl ester **3.3** (General procedure A1) to give **3.4** as a yellow oil which was not purified further (4.28 g, 87%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.25-7.35 (5H, m, Ph), 6.94 (2H, d, *J* = 8.5 Hz *H*ArO), 6.65-6.70 (3H, m, N*H* and *H*ArO), 5.62 (1H, d, *J* = 7.9 Hz, N*H*) 5.03 (2H, s, PhCH_2), 4.51-4.56 (1H, m, C*H*CH_2ArO), 4.42-4.46 (1H, m, C*H*$CH_2CH(CH_3)_2$), 3.65 (3H, s, OCH_3), 2.90-2.98 (2H, m, CHCH_2ArO), 1.50-1.59 (2H, m, $CHCH_2CH(CH_3)_2$ and CHC*H*$HCH(CH_3)_2$), 1.43-1.46 (1H, m, CHCH*H*$CH(CH_3)_2$), 0.82-0.95 (6H, m, 2 x CH_3).

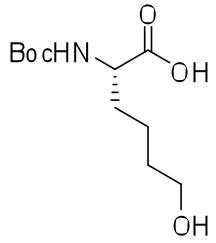
(*S*)-2-[(*S*)-2-Benzyloxycarbonylamino-3-(4-hydroxy-phenyl)-propionylamino]-4-methyl-pentanoic acid (3.5).



3.5

Methyl ester **3.4** (4.20 g, 9.4 mmol) was hydrolysed under aqueous basic conditions (General procedure B). The crude product was recrystallized from ethyl acetate to give **3.5** as a white solid (3.75 g, 92 %). ^{1}H NMR (500 MHz in $(CD_3)_2SO$): *δ* 9.31 (1H, br s, COO*H*), 8.22 (1H, d, *J* = 7.9 Hz, N*H*), 7.35-7.41 (5H, m, Ph), 7.10-7.13 (2H, m, *H*ArO), 6.67-6.69 (2H, m, *H*ArO), 4.93-4.97 (2H, s, PhCH_2), 4.21–4.31 (2H, m, C*H*CH_2ArO and C*H*$CH_2CH(CH_3)_2$), 3.61 (1H, br s, ArO*H*), 2.90-2.96 (1H, m, CHC*H*HArO), 2.63-2.67 (1H, m, CHCH*H*ArO), 1.68–1.71 (1H, m, $CHCH_2CH(CH_3)_2$), 1.54-1.60 (2H, m, CHCH_2CH$(CH_3)_2$), 0.88 (3H, d, *J*= 6.3 Hz, CH_3), 0.84 (3H, d, *J*= 6.5 Hz, CH_3).

(2*S*)-*tert*-Butoxycarbonylamino-6-hydroxyhexanoic acid (3.7).[2]



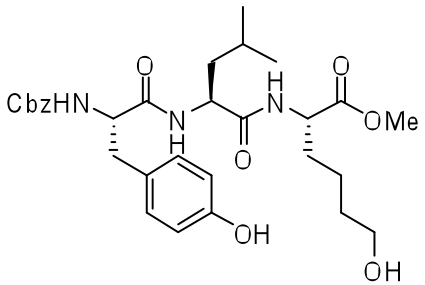
3.7

Diazotization of *N*-Boc-lysine **3.6** (31.8 g, 129.3 mmol) (General procedure I1) gave the crude product that was purified by flash column chromatography on silica gel and elution with ethyl acetate, petroleum spirit, HOAc (70:29:1) to give **3.7** (10.5 g, 33%) as a white solid. ^{1}H NMR (CD_3OD, 500 MHz): 4.90 (1H, br s,), 4.06 (1H, dd, J = 4.8, 9.0 Hz, C*H*$CH_2CH_2CH_2CH_2OH$), 3.55 (2H, t, J = 6.4 Hz, $CHCH_2CH_2CH_2CH_2OH$), 1.79-1.82 (1H, m, CHC*H*$HCH_2CH_2CH_2OH$), 1.64-1.68 (1H, m, $CHCHHCH_2CH_2CH_2OH$), 1.51-1.58 (2H, m, $CHCH_2CH_2CH_2CH_2OH$), 1.44-1.49 (11H, m, $C(CH_3)_3$ and $CHCH_2CH_2$ CH_2CH_2OH).

Lit cit: [2]Glenn, M. P.; Pattenden, L. K.; Reid, R. C.; Tyssen, D. P.; Tyndall, J. D. A.; Birch, C. J.; Fairlie, D. P. *J. Med. Chem*., **2002**, *45*, 371-381.

^{1}H NMR ($CDCl_3$, 300 MHz): 5.21 (br d, 1H); 4.28 (m, 1H); 3.66 (t, 2H); 1.41 (s, 9H); 1.90 to 1.37 (m, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz): 175.3, 156.5, 80.3, 62.1, 53.7, 32.4, 32.3, 28.5, 22.0.

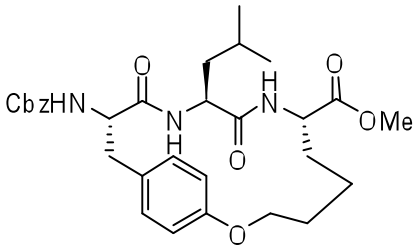
(*S*)-2-{(*S*)-2-[(*S*)-2-Benzyloxycarbonylamino-3-(4-hydroxy-phenyl)-propionylamino] -4-methyl-pentanoylamino}-6-hydroxy-hexanoic acid methyl ester (3.9).



3.9

Carboxylic acid **3.5** (5.02 g, 11.6 mmol) was coupled with Amine hydrochloride salt **3.8** and (General procedure A1). The crude product was recrystallized from ethyl acetate to give .**3.9** as a white solid (5.73 g, 87 %). ^{1}H NMR (500 MHz, CD_3OD): δ 7.24-7.37 (5H, m, Ph), 7.03 (2H, app d, *J* = 8.5 Hz, *H*ArO), 6.67 (2H, app d, *J* = 8.5 Hz, *H*Ar), 4.97-5.06 (2H, m, OC*H*$_2$Ar), 4.41-4.46 (1H, m, C*H*CH$_2$CH(CH$_3$)$_2$), 4.32-4.38 (2H, m, C*H*CH$_2$ArO and C*H*CO$_2$CH$_3$), 3.69 (3H, s, OC*H*$_3$), 3.50-3.55 (2H, m, C*H*$_2$OH), 2.98-3.03 (1H, m CHC*H*HAr), 2.73-2.77 (1H, m CHCH*H*Ar), 1.77-1.87 (1H, m, CHCH$_2$C*H*(CH$_3$)$_2$), 1.66-1.75 (1H, m, OCH$_2$C*H*HCH$_2$CH$_2$), 1.60-1.64 (1H, m, OCH$_2$CH*H*CH$_2$CH$_2$), 1.51-1.58 (4H, m, CHC*H*$_2$CH(CH$_3$)$_2$ and OCH$_2$CH$_2$C*H*$_2$CH$_2$), 1.38-1.48 (2H, m, OCH$_2$CH$_2$CH$_2$C*H*$_2$), 0.93 (3H, d, *J* = 6.5 Hz, C*H*$_3$), 0.90 (3H, d, *J* = 6.5 Hz, C*H*$_3$). ^{13}C NMR (75 MHz CD_3OD): δ 173.5, 173.0, 157.1, 156.1, 137.0, 130.4, 128.4, 128.0, 127.8, 127.5, 115.2, 66.5, 62.4, 61.5, 56.7, 52.7, 40.9, 31.9, 31.1, 24.6, 22.4, 22.1, 21.3. HRMS (ES) 572.2984 (MH^+); $C_{30}H_{42}N_3O_8$ requires 572.2972.

(7*S*,10*S*,13*S*)-13-Benzyloxycarbonylamino-10-isobutyl-9,12-dioxo-2-oxa-8, 11-diaza bicyclo[13.2.2]nonadeca-1(18),15(19),16-triene-7-carboxylic acid methyl ester (3.11).

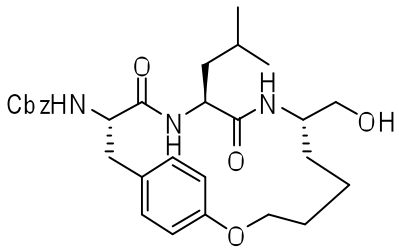


3.11

Intramolecular nucleophilic substitution of **3.10** (7.0 g, 0.01 mol) was conducted (General procedure P). Recrystallisation of the product from ethyl acetate and pentane gave **3.11** as a white solid (3.87 g, 70%). Mp 274-276 ºC. ^{1}H NMR (500 MHz, C_5D_5N): δ 9.44 (1H, d, *J* = 9.0 Hz, N*H*), 8.88 (1H, d, *J* = 8.0 Hz, N*H*), 8.52 (1H, d, *J* = 8.0 Hz, N*H*), 7.33 (2H, m, Ph), 7.18-7.23 (5H, m, Ph and *H*ArO), 6.86 (2H, app d, *J* = 8.5 Hz, *H*ArO), 5.25 (2H, br s, PhC*H*$_2$), 5.06-5.12 (1H, m C*H*CH$_2$ArO), 4.83-4.88 (1H, m, C*H*CH$_2$CH(CH$_3$)$_2$), 3.95 (1H, dd, *J* = 14.0 and 7.0 Hz, C*H*CO$_2$CH$_3$), 4.22-4.26 (1H, m, ArOC*H*H), 3.41-3.99 (1H, m,

ArOCH*H*), 3.54 (3H, s, OCH_3), 3.22 (1H, dd, *J* = 13.0 and 6.0 Hz, CHC*H*HArO), 3.14 (1H, app t, *J* = 12.0 and 12.0 Hz, CHCH*H*ArO), 1.76-1.82 (4H, m, $CHCH_2CH(CH_3)_2$, $CHCHHCH(CH_3)_2$, $ArOCH_2CHH$ and $CHHCHCO_2CH_3$), 1.60-1.67 (2H, m, $CH_2CHCO_2CH_3$ and $CHCH_2CH(CH_3)_2$), 1.41-1.52 (1H, m, $CH_2CHHCHCO_2CH_3$), 1.21-1.32 (2H, m, $ArOCH_2CHH$ and $CH_2CHHCHCO_2CH_3$), 0.72 (3H, d, *J* = 6.5 Hz, CH_3), 0.68 (3H, d, *J* = 6.5 Hz, CH_3). ^{13}C NMR (75 MHz in $CDCl_3$): δ 172.5, 170.8, 169.7, 157.1, 155.5, 136.3, 130.1, 128.5, 128.2, 128.1, 127.9, 115.7, 66.8, 66.7, 57.1, 52.5, 51.7, 51.2, 43.3, 39.0, 31.5, 28.0, 24.5, 22.9, 22.4, 21.2. HRMS (ES) 554.2859 (MH^+); $C_{30}H_{40}N_3O_7$ requires 554.2866.

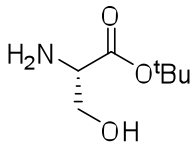
((7*S*,10*S*,13*S*)-7-Hydroxymethyl-10-isobutyl-9,12-dioxo-2-oxa-8,11-diaza bicycle [13.2.2] nonadeca-1(18),15(19),16-trien-13-yl)-carbamic acid benzyl ester (3.12).



3.12

The macrocyclic methyl ester **3.11** (1.50 g, 2.7 mmol) was reduced (General procedure G2) and the crude product was recrystallisation from ethyl acetate to give **3.12** as a white solid (1.38 g, 97%). ^1H NMR (500 MHz, (CD_3OD): *δ* 7.26–7.36 (3H, m, Ph), 7.05 (2H, m, *H*ArO), 6.79 (2H, m, *H*ArO), 5.05-5.13 (1H, m, $PhCH_2$), 4.29–4.34 (2H, m, $CHCH_2ArO$ and ArOC*H*H), 4.05–4.10 (1H, m, ArOCH*H*), 3.96–4.02 (1H, m, $CHCH_2CH(CH_3)_2$), 3.79 (1H, m, $CHCH_2OH$), 3.32 (2H, m, CH_2OH), 2.98 (1H, m, CHC*H*HArO), 2.69 (1H, m, CHCH*H*ArO), 1.75–1.84 (2H, m, CH_2CHCH_2OH), 1.51–1.56 (3H, m, $CHCH_2CH(CH_3)_2$ and $CHCH_2CH(CH_3)_2$), 1.22–1.42 (4H, m, $ArOCH_2CH_2$ and $CH_2CH_2CHCH_2OH$), 0.83-0.87 (6H, m, 2 x CH_3).

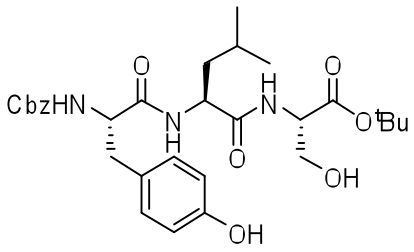
(*S*)-2-Amino-3-hydroxy-propionic acid *tert*-butyl ester (3.14).



3.14

N-Cbz-Ser-O^{t}Bu **3.13** (3 g, 10.2 mmol) was hydrogenated (General procedure D) to give product **3.14** (1.6 g, 97%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.78-3.82 (1H, dd, *J* = 3.9 and 10.8 Hz, CHC*H*HOH), 3.64-3.68 (1H, dd, *J* = 5.9 and 10.7 Hz, CHCH*H*OH), 3.43-3.48 (1H, m, C*H*CH_2OH), 1.49 (9H, s, OC(CH_3)$_3$). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 173.3, 81.9, 64.4, 56.6, 28.2. MS (ES) 162.11 (MH^+); $C_7H_{16}NO_3$ requires 162.11.

(*S*)-2-{(*S*)-2-[(*S*)-2-Benzyloxycarbonylamino-3-(4-hydroxy-phenyl)-propionylamino] -4-methyl-pentanoylamino}-3-hydroxy-propionic acid tert-butyl ester (3.16).

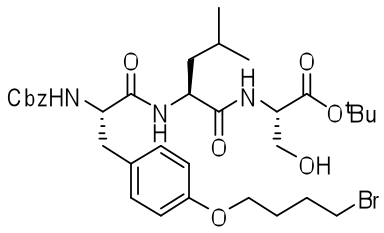


3.16

Ser-O^{t}Bu **3.14** (1.60 g, 9.9 mmol) was coupled with carboxylic acid **3.5** (General procedure A1) to give the crude product. The crude product was purified by flash chromatography on silica gel and elution with ethyl acetate/petroleum ether (3:2) as eluent to give **3.16** as a white solid (4.58 g, 78%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.64 (1H, d, *J* = 9.0 Hz, N*H*), 7.26-7.29 (5H, m, Ph), 7.23 (1H, d, *J* = 7.1 Hz, N*H*), 6.89 (2H, m, *J* = 7.9 Hz, Ar*H*), 6.64 (2H, m, *J* = 8.1Hz, Ar*H*), 6.03 (1H, d, *J* = 7.2 Hz, N*H*), 5.04 (1H, d, *J* = 12.5 Hz, PhC*H*H), 5.00 (1H, d, *J* = 12.5 Hz, PhCH*H*), 4.55-4.58 (2H, m, C*H*CH_2CH(CH_3)$_2$ and C*H*CH_2OH), 4.45 (1H, m, C*H*CH_2ArO), 3.82 (2H, s, CHCH_2OH), 2.85-2.94 (2H, m, CHCH_2ArO), 1.48-1.61 (3H, m, CHCH_2CH(CH_3)$_2$ and CHCH_2C*H*(CH_3)$_2$). 1.43 (9H, s, C(CH_3)$_3$), 0.82-0.87 (6H, m, CHCH_2CH(CH_3)$_2$). ^{13}C NMR (75 MHz CD_3OD): δ 172.5, 172.3, 169.5, 156.4, 155.5, 136.3, 130.6, 128.7, 128.4,

128.2, 115.9, 83.0, 67.3, 63.1, 57.0, 55.6, 52.3, 41.2, 28.3, 24.8, 22.9, 22.4. HRMS (ES) 594.2779 (MNa^+); $C_{30}H_{41}N_3NaO_8$ requires 594.2786.

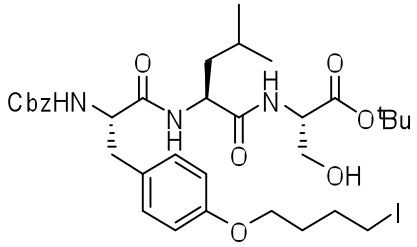
(*S*)-2-((*S*)-2-{(*S*)-2-Benzyloxycarbonylamino-3-[4-(4-bromo-butoxy)-phenyl] propionyl amino}-4-methyl-pentanoylamino)-3-hydroxy-propionic acid *tert*-butyl ester (3.18).



3.18

Tripeptide **3.16** (200 mg, 0.35mmol), potassium carbonate (72.5 mg, 0.52 mmol) and cesium carbonate (11.4 mg, 0.035 mmol) were dissolved in MeCN (10 ml). To this solution was added dibromobutane **3.17** (418 µl) and solution was stirred under reflux condition overnight. The reaction solution was concentrated and residue was partitioned between ethyl acetate and 1M HCl. The organic layer was washed by 1M HCl, distilled water, brine and dried over $MgSO_4$ before concentrated in *vacuo* to give the crude product **3.18** as a white crystal (252 mg) The crude product was purified by flash chromatography on silica gel and elution with ethyl acetate/petroleum ether (1:1) to give **3.18** as a yellow oil (131 mg, 53%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.30-7.38 (5H, m, Ph), 7.11 (2H, d, *J*= 8.5Hz, *H*ArO), 6.83 (2H, d, *J*= 8.5Hz, *H*ArO), 6.63 (1H, d, *J* = 7.8 Hz, N*H*), 5.56 (1H, d, *J* = 7.1 Hz, N*H*), 5.09 (2H, m, OCH_2Ar), 4.53-4.56 (2H, m, C*H*$CH_2CH(CH_3)_2$ and C*H*CH_2OH), 4.45-4.48 (1H, m, C*H*CH_2ArO), 3.91 (2H, m, ArOC$H_2CH_2CH_2CH_2$Br), 3.85 (CHCH_2OH), 3.45 (2H, ArOCH_2CH_2-CH_2CH_2Br), 3.01 (2H, m, CHCH_2Ar), 2.07 (2H, m, ArOC$H_2CH_2CH_2CH_2$Br), 1.92 (2H, m, ArOC$H_2CH_2CH_2CH_2$Br), 1.52-1.70 (3H, m, $CHCH_2CH(CH_3)_2$ and $CHCH_2CH(CH_3)_2$), 1.47 (9H, s, OC(CH_3)$_3$), 086-0.88 (6H, m, $CHCH_2CH(CH_3)_2$).^{13}C NMR (75 MHz CD_3OD): δ 172.0, 169.5, 158.2, 157.0, 136.2, 130.6, 130.4, 128.8, 128.5, 128.3, 128.2, 114.9, 82.9, 67.5, 67.0, 63.3, 56.6, 55.7, 52.2, 40.7, 36.9, 33.7, 29.7, 28.2, 24.9, 23.1, 22.2. HRMS (ES) 728.2540 (MNa^+); $C_{34}H_{48}BrN_3NaO_8$ requires 728.2517.

(*S*)-2-((*S*)-2-{(*S*)-2-Benzyloxycarbonylamino-3-[4-(4-iodo-butoxy)-phenyl]-propionyl amino}-4-methyl-pentanoylamino)-3-hydroxy-propionic acid *tert*-butyl ester (3.20).

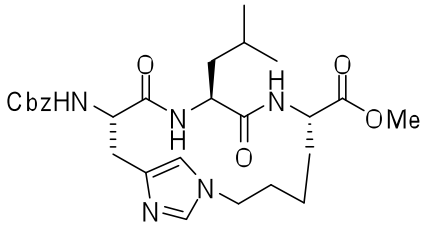


3.20

To a solution of alkyl bromide **3.18** (250 mg, 0.35 mmol) in acetone (4 ml) was added sodium iodide (80 mg, 0.53 mmol). The resulting solution was stirred overnight and concentrated in *vacuo*. The residue was partitioned between ethyl acetate and 1M HCl and the organic layer was washed by 1M HCl, distilled water, brine and dired over $MgSO_4$ and the solvent removed in vacuo to give **3.20** as a yellow oil (260 mg, 98%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.30-7.37 (5H, m, Ph), 7.12 (2H, d, *J*= 8.6 Hz, *H*ArO), 6.83 (2H, d, *J*= 8.6 Hz, *H*ArO), 6.53 (1H, d, *J* = 7.8 Hz, N*H*), 5.51 (1H, d, *J* = 7.0 Hz, N*H*), 5.09 (2H, m, OCH_2Ar), 4.52-4.55 (2H, m, C*H*$CH_2CH(CH_3)_2$ and C*H*CH_2OH), 4.41 (1H, m, C*H*CH_2ArO), 3.97 (2H, m, ArOC$H_2CH_2CH_2CH_2$Br), 3.90 (CHCH_2OH), 3.31 (2H, Ar$OCH_2CH_2CH_2CH_2$Br), 3.03 (2H, m, CHCH_2Ar), 2.03 (2H, m, Ar$OCH_2CH_2CH_2CH_2$Br), 1.89-1.94 (4H, m, Ar$OCH_2CH_2CH_2CH_2$Br and CHC$H_2CH(CH_3)_2$), 1.69 (1H, m, $CHCH_2CH(CH_3)_2$) and 1.52 (9H, s, $OC(CH_3)_3$), 0.92-0.94 (6H, m, $CHCH_2CH(CH_3)_2$). HRMS (ES) 776.2361 (MNa^+); $C_{34}H_{48}IN_3NaO_8$ requires 776.2378.

7.4: Experimental work described in chapter 4

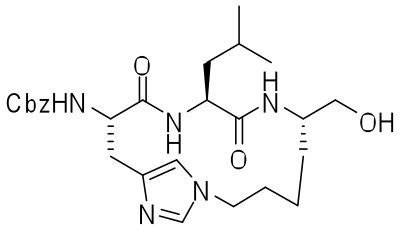
(6*S*,9*S*,12*S*)-12-Benzyloxycarbonylamino-9-isobutyl-8,11-dioxo-1,7,10,15-tetraaza-bicyclo[12.2.1] heptadeca-14(17),15-diene-6-carboxylic acid methyl ester (4.1a)



4.1a

Intramolecular lactamization of macrocyclic precursor **4.15** (1.22 g, 2.24 mmol) (General procedure O2) gave the macrocycle and the crude product was purified by chromatography on silica gel and elution with methanol/dichloromethane (1:20) to give the **4.1a** as a white solid (620 mg, 52%). ^{1}H NMR (CD_3OD, 500 MHz): δ 8.56 (1H, s, 2-Ar*H*), 7.35-7.37 (5H, m, Ph), 7.17 (1H, s, 5-Ar*H*), 5.16 (1H, d, *J* = 12.2, OC*H*HAr), 5.08 (1H, d, *J* = 12.5 Hz, OCH*H*Ar), 4.63-4.65 (1H, m, C*H*$CH_2CH(CH_3)_2$), 4.53-4.55 (1H, m, C*H*CH_2Ar), 4.34-4.37 (1H, m, C*H*$CH_2CH_2CH_2CH_2$Ar), 4.23-4.26 (1H, m, $CHCH_2CH_2CH_2$C*H*HAr), 4.10-4.13 (1H, m, $CHCH_2CH_2CH_2$CH*H*Ar), 3.71 (3H, s, OCH_3), 3.12 (2H, m, CHCH_2Ar), 2.12-2.15 (1H, m, $CHCH_2CH_2$C*H*HCH_2Ar), 1.95-1.99 (1H, m, $CHCH_2CH_2$CH*H*CH_2Ar), 1.63-1.73 (3H, m, CH$CH_2CH(CH_3)_2$ and C*H*CH_2C*H*$(CH_3)_2$), 1.51-1.54 (2H, m, CH$CH_2CH_2CH_2CH_2$Ar), 1.21-1.26 (1H, m, $CHCH_2$C*H*HCH_2CH_2Ar), 1.08-1.12 (1H, m, $CHCH_2$C*H*HCH_2CH_2Ar), 0.94 (3H, d, *J* = 6.4 Hz, CH(C$H_3)_2$), 0.89 (3H, d, *J* = 6.4 Hz, CH(C$H_3)_2$). ^{13}C NMR ($CDCl_3$, 75 MHz): 173.0, 172.4, 170.1, 156.1, 137.8, 137.7, 137.0, 129.0, 128.5, 128.4, 116.7, 66.1, 54.0, 52.6, 51.5, 49.4, 46.3, 41.7, 41.0, 31.0, 30.6, 28.1, 24.7, 23.2, 23.0, 21.3. HRMS (ES) 528.2830 (MH^+); $C_{27}H_{38}N_5O_6$ requires 528.2822.

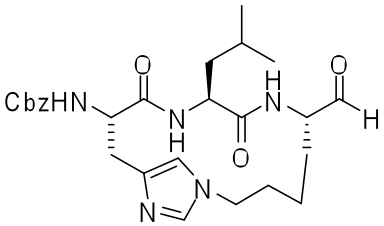
((6*S*,9*S*,12*S*)-6-Hydroxymethyl-9-isobutyl-8,11-dioxo-1,7,10,15-tetraaza-bicyclo [12.2.1] heptadeca-14(17), 15-dien-12-yl)-carbamic acid benzyl ester (4.1b).



4.1b

The macrocyclic methyl ester **4.1a** (200 mg, 0.378 mmol) was reduced (General procedure G2) and the crude product was recrystallised from ethanol to give **4.1b** as a white solid (185 mg, 98%). ^{1}H NMR (DMSO, 500 MHz): δ 8.15 (1H, s, 2-Ar*H*), 7.95 (1H, d, *J* = 8.6 Hz, NH), 7.78 (1H, d, *J* = 9.0 Hz, NH), 7.50 (1H, d, *J* = 7.1 Hz, NH), 7.29-7.35 (5H, m, Ph), 7.13 (1H, s, 5-Ar*H*), 4.97-5.02 (2H, m, OC*H*$_2$Ar), 4.59 (1H, m, CH$_2$O*H*), 4.55-4.58 (C*H*CH$_2$Ar), 4.27-4.29 (1H, m, C*H*CH$_2$CH(CH$_3$)$_2$), 3.94 (2H, m, CHCH$_2$CH$_2$CH$_2$C*H*$_2$Ar), 3.78 (1H, m, C*H*CH$_2$CH$_2$CH$_2$CH$_2$Ar), 3.21-3.25 (1H, m, C*H*HOH), 3.14-3.18 (1H, m, CH*H*OH), 3.07-3.10 (1H, m, CHC*H*HAr), 2.65-2.67 (1H, m, CHCH*H*Ar), 1.85-1.97 (2H, m, CHCH$_2$CH$_2$C*H*$_2$CH$_2$Ar), 1.53-1.55 (1H, m, CHC*H*HCH$_2$C*H*$_2$CH$_2$Ar), 1.30-1.45 (3H, m, CHC*H*$_2$CH(CH$_3$)$_2$ and CHCH$_2$C*H*(CH$_3$)$_2$), 1.15-1.18 (1H, m, CHCH*H*CH$_2$C*H*$_2$CH$_2$Ar), 1.05-1.09 (1H, m, CHCH$_2$C*H*HCH$_2$CH$_2$Ar), 0.94-0.96 (1H, m, CHCH$_2$CH*H*CH$_2$CH$_2$Ar), 0.80-0.86 (6H, m, CH(C*H*$_3$)$_2$). ^{13}C NMR (CDCl$_3$, 75 MHz): δ 171.7, 160.6, 156.1, 137.9, 137.8, 134.0, 129.0, 128.4, 128.3, 118.9, 66.0, 64.8, 51.7, 53.1, 51.7, 48.0, 42.3, 41.0, 30.5, 28.7, 27.1, 24.8, 23.2. HRMS (ES) 500.2870 (MH$^+$); $C_{26}H_{38}N_5O_5$ requires 500.2873.

((6*S*,9*S*,11*S*)-6-Formyl-9-isobutyl-8,11-dioxo-1,7,10,15-tetraaza-bicyclo [12.2.1] heptadeca-14(17),15-dien-12-yl)-carbamic acid benzyl ester (4.1c).[1]



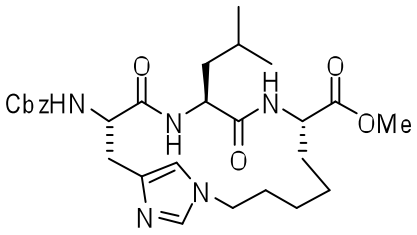
4.1c

The macrocyclic alcohol **4.1b** (30 mg, 0.06 mmol) was oxidised (General procedure H3) and the crude product was purified by chromatography on silica gel and elution with methanol/dichloromethane (1:99) to give **4.3c** as a white solid (20 mg, 67%). ^{1}H NMR (300 MHz, CDCl$_3$) δ 9.54 (s, 1H), 7.47 – 7.29 (m, 6H), 6.64 (s, 1H), 6.38 (d, *J* = 8.7 Hz, 1H), 6.23 (d, *J* = 8.0 Hz, 1H), 6.17 (d, *J* = 7.3 Hz, 1H), 5.12 (q, *J* = 12.2 Hz, 2H), 4.63

[1] Oxidation of **4.1b** to **4.1c** was carried out by Ashok Pehere at the University of Adelaide using commercially available Dess-Martin periodinane.

(dd, *J* = 21.2, 11.1 Hz, 2H), 4.30 (dd, *J* = 12.6, 5.3 Hz, 1H), 4.09 – 3.92 (m, 1H), 3.92 – 3.77 (m, 1H), 3.08 – 2.91 (m, 2H), 2.16 – 1.86 (m, 3H), 1.85 – 1.50 (m, 4H), 1.18 – 1.00 (m, 2H), 0.93 (d, *J* = 4.2 Hz, 3H), 0.88 (d, *J* = 3.7 Hz, 3H).^{13}C NMR (151 MHz, $(CD_3)_2SO$) δ 200.96, 172.19, 171.62, 157.73, 136.82, 135.08, 128.31, 127.85, 127.74, 118.65, 65.59, 55.68, 54.32, 52.71, 51.04, 41.03, 35.07, 28.97, 24.10, 22.71, 22.21, 13.91. HRMS (ES) 498.2711 (MH^+); $C_{27}H_{38}N_5O_6$ requires 498.2716.

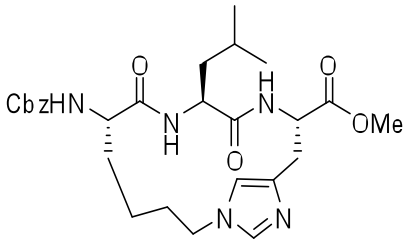
(7*S*,10*S*,13*S*)-13-Benzyloxycarbonylamino-10-isobutyl-9,12-dioxo-1,8,11,16-tetraaza-bicyclo[13.2.1] octadeca-15(18),16-diene-7-carboxylic acid methyl ester (4.2a).



4.2a

Intramolecular lactamization of macrocyclic precursor **4.36** (335 mg, 0.6 mmol) (General procedure O2) gave the macrocycle and the crude product was purified by chromatography on silica gel and elution with methanol/dichloromethane (1:10) to give **4.2a** as a white solid (160 mg, 50%) ^{1}H NMR (CD_3OD, 500 MHz): δ 7.53 (1H, s, 2-Ar*H*), 7.33-7.35 (5H, m, Ph), 6.79 (1H, s, 5-Ar*H*), 5.10 (1H, m, OCH_2Ar), 4.50-4.52 (1H, m, C*H*CH_2Ar), 4.42-4.45 (2H, m, C*H*$CH_2CH(CH_3)_2$ and C*H*$CH_2CH_2CH_2CH_2$ CH_2Ar), 4.01 (1H, m, $CHCH_2CH_2CH_2CH$HAr), 3.85 (1H, m, $CHCH_2CH_2CH_2CH$*H*Ar), 3.68 (3H, s, OCH_3), 2.98 (2H, m, CHCH_2Ar), 1.82-1.94 (2H, $CHCH_2CH_2CH_2CH$HCH_2Ar and C*H*$CH_2CH_2CH_2CH_2CH_2$Ar), 1.60-1.75 (4H, $CHCH_2CH_2CH_2CH$*H*CH_2Ar, CHC$H_2CH_2CH_2CH_2CH_2$Ar and $CHCH_2CH(CH_3)_2$), 1.51 (2H, m, CHC$H_2CH(CH_3)_2$), 1.35-1.40 (2H, m, CHCH_2CH_2CHHCH_2CH_2Ar), 1.21 (1H, m, CHCH_2CH_2CHHCH_2CH_2Ar), 0.90-0.94 (6H, m, $CHCH_2CH(CH_3)_2$). ^{13}C NMR (CD_3OD, 75 MHz): 173.1, 173.0, 171.4, 156.8, 137.3, 137.0, 136.1, 128.3, 127.8, 127.7, 117.5, 66.4, 54.5, 51.8, 51.5, 51.1, 50.4, 46.3, 41.8, 30.4, 30.3, 29.9, 24.9, 24.4, 24.3, 22.2, 21.3. HRMS (ES) 542.2979 (MH^+); $C_{27}H_{38}N_5O_6$ requires 542.2979.

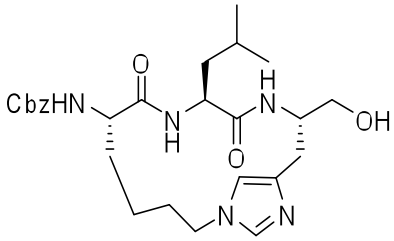
(6*S*,9*S*,12*S*)-6-Benzyloxycarbonylamino-9-isobutyl-7,10-dioxo-1,8,11,15-tetraaza bicycle [12.2.1] heptadeca-14(17),15-diene-12-carboxylic acid methyl ester (4.3a)



4.3a

Acidolysis of pseudo-tripeptide **4.39** (430 mg, 0.62 mmol) in the presence of trifloroacetic acid (General procedure N) followed by intramolecular lactamization of macrocyclic precursor **4.38** (General procedure O2) gave the macrocycle. The crude product was purified by chromatography on silica gel and elution with methanol/ dichloromethane (1:20) to give **4.3a** as a white solid (167 mg, 51%). ^{1}H NMR (CD_3OD, 500 MHz): δ 7.57 (1H, s, 2-Ar*H*), 7.29-7.33 (5H, m, Ph), 6.81 (1H, s, 5-Ar*H*), 5.06 (2H, s, OCH_2Ph), 4.84-4.86 (1H, m, C*H*CH_2Ar), 4.52 (1H, m, C*H*$CH_2CH_2CH_2CH_2$Ar), 4.05-4.07 (1H, dd, *J* = 4.2 and 9.7 Hz, C*H*$CH_2CH(CH_3)_2$), 3.97 (2H, m, $CHCH_2CH_2CH_2CH_2$Ar), 3.76 (3H, s, OCH_3), 3.17-3.20 (1H, dd, *J* = 2.6 and 15.0Hz, CHCH*H*Ar), 2.80 (1H, m, CHC*H*HAr), 1.78-1.81 (1H, m, $CHCH_2CH_2CHCH_2$Ar), 1.55-1.70 (5H, m, $CHCH_2CH_2CHCH_2$Ar, $CHCH_2CH_2CH_2CH_2$Ar, $CHCH_2CH(CH_3)_2$ and $CHCHCH(CH_3)_2$), 1.50-1.55 (1H, m, $CHCHCH(CH_3)_2$), 1.09-1.11 (1H, m, $CHCHCH(CH_3)_2$), 0.96 (3H, d, *J* = 6.4Hz, $CHCH_2CH(CH_3)_2$), 0.89 (3H, d, *J* = 6.4Hz, $CHCH_2CH(CH_3)_2$). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 172.7, 172.3, 171.8, 156.7, 137.0, 136.8, 136.2, 128.3, 127.8, 127.7, 117.5, 66.4, 54.1, 51.8, 51.4, 51.2, 45.8, 41.1, 31.3, 30.3, 28.3, 24.5, 21.8, 21.6, 21.3. HRMS (ES) 528.2799 (MH^+); $C_{27}H_{38}N_5O_6$ requires 528.2822.

((6*S*,9*S*,12*S*)-12-Hydroxymethyl-9-isobutyl-7,10-dioxo -1,8,11,15-tetraaza-bicyclo [12.2.1] heptadeca-14(17), 15-dien-6-yl)-carbamic acid benzyl ester (4.3b).

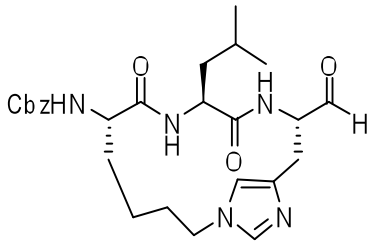


4.3b

The macrocyclic methyl ester **4.1a** (160 mg, 0.30 mmol) was reduced (General procedure G2) and crude product was purified by chromatography on silica gel and elution with methanol/dichloromethane (1:10) to give **4.3b** as a white solid (116 mg,72%). ^{1}H NMR (CD_3OD, 500 MHz): δ 8.44 (1H, s, 2-Ar*H*), 7.29-7.34 (5H, m, Ph), 7.08 (1H, s, 5-Ar*H*), 5.06 (2H, s, OCH_2Ar), 4.40 (1H, m, C*H*$CH_2CH_2CH_2CH_2$Ar), 4.21 (1H, m, C*H*CH_2Ar), 4.14 (2H, m, $CHCH_2CH_2CH_2CH_2$Ar), 4.06 (1H, dd, *J* = 4.7 and 10.2Hz, C*H*$CH_2CH(CH_3)_2$), 3.58 (2H, m, CH_2OH), 2.98 (1H, d, *J* = 14.1 Hz, CHC*H*HAr), 2.65 (1H, m, CHCH*H*Ar), 1.85 (1H, m, $CHCH_2CH_2CHHCH_2$Ar), 1.71-1.73 (2H, m, $CHCH_2CH_2CHHCH_2$Ar and $CHCH_2CHHCH_2CH_2$Ar), 1.60-1.70 (3H, $CHCH_2CH$*H* CH_2CH_2Ar, $CHCH_2CH(CH_3)_2$ and $CHCHHCH(CH_3)_2$), 1.54 (1H, m, $CHCHHCH_2CH_2CH_2$Ar), 1.48 (1H, m, $CHCHHCH_2CH_2CH_2$Ar), 1.18 (1H, m, CHCH*H* $CH(CH_3)_2$), 0.89-0.93 (6H, m, $CHCH_2CH(CH_3)_2$). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 171.9, 171.2, 160.6, 156.0, 138.8, 137.8, 129.0, 128.5, 128.4, 123.1, 116.6, 65.9, 64.7, 51.3, 49.7, 47.7, 42.6, 40.8, 30.7, 24.8, 23.3, 23.1, 22.2. HRMS (ES) 500.2850 (MH^+). $C_{26}H_{38}N_5O_5$ requires 500.2867.

((7*S*,9*S*,12*S*)-12-Formyl-9-isobutyl-7,10-dioxo-1,8,11,15-tetraaza-bicycl o[12.2.1] heptadeca-14(17),15-dien-6-yl)-carbamic acid benzyl ester (4.3c).[2]

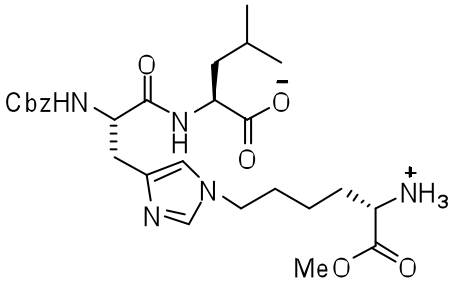
[2] Oxidation of **4.3b** to **4.3c** was carried out by Ashok Pehere at the University of Adelaide using commercially available Dess-Martin periodinane.



4.3c

The macrocyclic alcohol **4.3b** (20 mg, 0.04 mmol) was oxidised (General procedure H3) and the crude product was purified by chromatography on silica gel and elution with methanol/dichloromethane (1:99) to give **4.3c** as a white solid (10 mg, 50%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.71 (s, 1H), 7.34 (m, *J* = 23.4, 4.8 Hz, 6H), 6.83 (d, *J* = 6.0 Hz, 1H), 6.67 (s, 1H), 6.30 (d, *J* = 7.2 Hz, 1H), 5.53 (d, *J* = 7.2 Hz, 1H), 5.10 (d, *J* = 23.5 Hz, 2H), 4.80 (dd, *J* = 11.9, 8.0 Hz, 1H), 4.46 (dd, *J* = 14.8, 8.3 Hz, 1H), 4.09 (dd, *J* = 11.3, 7.7 Hz, 1H), 3.89 (m, 2H), 3.39 (dd, *J* = 15.2, 4.1 Hz, 1H), 2.81 (dd, *J* = 14.9, 8.9 Hz, 1H), 2.17 – 1.93 (m, 2H), 1.90 – 1.42 (m, 4H), 1.40 – 1.03 (m, 3H), 0.96 – 0.77 (m, 6H).^{13}C NMR (151 MHz, $CDCl_3$) δ 198.25, 171.90, 170.37, 155.58, 136.96, 136.60, 135.93, 128.54, 128.22, 128.02, 116.86, 66.99, 58.52, 54.32, 51.56, 46.25, 41.55, 31.97, 29.70, 24.67, 22.69, 22.11, 14.12. HRMS (ES) 498.2716 (MH^+); $C_{26}H_{36}N_5O_5$ requires 498.2711.

(*S*)-2-Amino-6-{4-[(*S*)-2-benzyloxycarbonylamino-2-((*S*)-1-carboxy-3-methyl-butyl carbamoyl)-ethyl]-imidazol-1-yl}-hexanoic acid methyl ester (4.15).

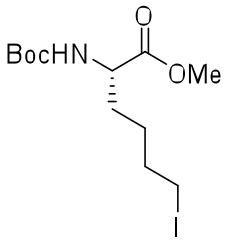


4.15

Acidolysis of pseudo-tripeptide **4.21** (1.6 g, 2.3 mmol) in the presence of trifloroacetic acid (General procedure N) to give **4.15** (1.22 g, 99%) as a colourless oil which was used

without purification. ^{1}H NMR for major product from mixture (CD_3OD, 500 MHz): δ 8.78 (1H, s, 2-Ar*H*), 7.40 (1H, s, 5-Ar*H*), 7.34-7.36 (5H, m, Ph), 5.08 (2H, m, OCH_2Ar), 4.49-4.52 (1H, m, C*H*CH_2Ar), 4.40-4.43 (1H, m, C*H*CH_2CH(CH_3)$_2$), 4.15-4.18 (2H, m, CHCH_2CH_2CH_2CH_2Ar), 4.03-4.06 (1H, m, C*H*CH_2CH_2CH_2CH_2 Ar), 3.83 (3H, s, OCH_3), 3.29-3.31 (1H, dd, , *J* = 6.8 and 15.4 Hz, CHC*H*HAr), 3.03-3.06 (1H, dd, *J* = 7.2 and 15.6 Hz, CHCH*H*Ar), 1.85-2.00 (4H, CHCH_2CH_2CH_2CH_2Ar and CHCH_2CH_2CH_2CH_2Ar), 1.62-1.75 (3H, m, CHCH_2C*H*(CH_3)$_2$ and CHCH_2CH(CH_3)$_2$), 1.41-1.49 (2H, m, CHCH_2CH_2CH_2CH_2Ar), 0.95 (3H, d, *J* = 6.3Hz, CHCH_2CH(CH_3)$_2$), 0.91 (3H, d, *J* = 6.1Hz, CHCH_2CH(CH_3)$_2$). ^{13}C NMR (CD_3OD, 75 MHz): δ 174.8, 171.1, 169.6, 158.5, 136.9, 134.5, 130.4, 127.8, 125.1, 120.2, 114.1, 66.7, 53.8, 52.5, 51.0, 45.8, 40.1, 29.7, 29.2, 27.6, 24.8, 22.2, 21.7, 20.5, 20.3. HRMS (ES) 546.2914 (MH^+). $C_{27}H_{40}N_5O_7$ requires 546.2928.

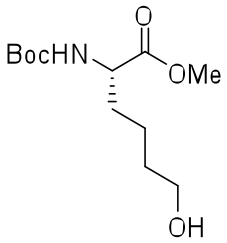
(*S*)-2-*tert*-Butoxycarbonylamino-6-iodo-hexanoic acid methyl ester (4.17)



4.17

Primary alcohol **4.18** (3.2 g, 12.2 mol) was converted to the iodide (General procedure L1). The crude product was purified by chromatography on silica gel and elution with ethyl acetate/petroleum ether (1:5) to give **4.17** (2.94 g, 66%): ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.03 (1H, d, *J* = 14.1 Hz, N*H*), 4.29-4.30 (1H, m, C*H*CH_2CH_2CH_2CH_2I), 3.75 (3H, s, OCH_3), 3.16-3.18 (2H, m, CHCH_2CH_2CH_2CH_2I), 1.81-1.84 (3H, CHCH_2CH_2CH_2CH_2I and CHC*H*HCH_2CH_2CH_2I), 1.60-1.63 (CHCH*H*CH_2CH_2CH_2I), 1.44-1.47 (11H, m, CHCH_2CH_2CH_2CH_2I and C(CH_3)$_3$) ^{13}C NMR ($CDCl_3$, 75 MHz): δ 173.4, 155.5, 80.2, 53.3, 52.6, 32.9, 31.9, 28.5, 26.4, 6.4. HRMS (ES) 372.0687 (MH^+). $C_{12}H_{23}NO_4I$ requires 372.0672.

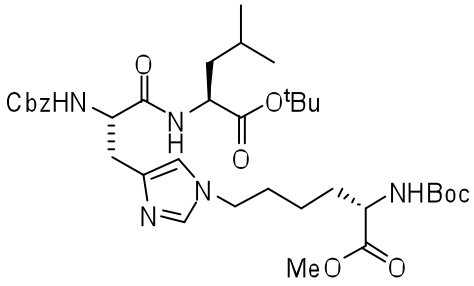
(*S*)-2-*tert*-Butoxycarbonylamino-6-hydroxy-hexanoic acid methyl ester (4.18).



4.18

Carboxylic acid **4.12** (2.3 g, 9.3 mmol) was esterified with methyl iodide (General procedure J1) and the crude product purified by chromatography on silica gel and elution with cyclohexane/ethyl acetate (8:2) to give **4.18** as a yellow oil (1.58 g, 65%): ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.15 (1H, N*H*) 4.22-4.26 (1H, m, C*H*$CH_2CH_2CH_2CH_2OH$), 3.69 (3H, s, OCH_3), 3.56-3.59 (2H, m, $CHCH_2CH_2CH_2CH_2OH$), 1.74-1.79 (1H, m, CHC*H*$HCH_2CH_2CH_2OH$), 1.62-1.65 (H, $CHCHHCH_2CH_2CH_2OH$), 1.50-1.55 (2H, m, $CHCH_2CH_2CH_2CH_2OH$), 1.38-1.42 (1H, m, $CHCH_2CH_2CH_2CH_2OH$ and $OC(CH_3)_3$). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 173.6, 162.8, 80.1, 62.5, 53.5, 52.5, 36.7, 32.7, 32.3, 31.7, 28.5, 21.8. HRMS (ES) 262.1658 (MH^+). $C_{12}H_{24}NO_5$ requires 262.1654.

6-{4-[(*S*)-2-Benzyloxycarbonylamino-2-((*S*)-1-*tert*-butoxycarbonyl-3-methyl-butyl carba moyl)-ethyl]-imidazol-1-yl}-2-*tert*-butoxycarbonylamino-hexanoic acid methyl ester (4.21)

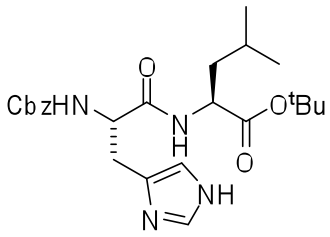


4.21

The imidazole ring of dipeptide **4.22** (3.5 g, 7.6 mmol) was alkylated with alkyl halide **4.17** (General procedure M2) and the crude product was purified by column chromatography on silica gel by elution with ethyl acetate/petroleum ether (6:1) to give **4.21** as a yellow oil (2.66 g, 50%). ^{1}H NMR (CD_3OD, 500 MHz): δ 7.58 (1H, s, 2-Ar*H*),

7.29-7.32 (5H, m, Ph), 6.91 (1H, s, 5-Ar*H*), 5.04 (2H, m, OC*H*$_2$Ar), 4.38-4.41 (1H, m, C*H*CH$_2$Ar), 4.31-4.32 (1H, m, C*H*CH$_2$CH(CH$_3$)$_2$), 4.08-4.12 (1H, m, C*H*CH$_2$CH$_2$CH$_2$CH$_2$Ar), 3.90-3.93 (2H, m, CHCH$_2$CH$_2$CH$_2$C*H*$_2$Ar), 3.68 (3H, s, OC*H*$_3$), 3.02-3.05 (1H, dd, *J* = 4.8 and 14.9 Hz, CHC*H*HAr) 2.82-2.85 (1H, dd, *J* = 9.2 and 14.9 Hz, CHCH*H*Ar), 1.72-1.80 (3H, m, CHCH$_2$CH$_2$C*H*$_2$CH$_2$Ar and CHCH$_2$C*H*(CH$_3$)$_2$), 1.62-1.65 (2H, m, CHC*H*$_2$CH(CH$_3$)$_2$), 1.57-1.62 (2H, m, CHC*H*$_2$CH$_2$CH$_2$CH$_2$Ar), 0.94 (3H, d, *J* = 6.4Hz, CH$_3$), 0.89 (3H, d, *J* = 6.4Hz, CH$_3$). ^{13}C NMR (CDCl$_3$, 75 MHz): δ 173.6, 172.8, 172.0, 156.9, 137.0, 136.8, 136.7, 128.3, 127.8, 127.7, 117.4, 110.4, 81.4, 79.4, 66.4, 55.0, 53.6, 51.7, 51.4, 46.7, 40.4, 30.9, 30.5, 30.2, 27.5, 27.1, 24.7, 22.6, 22.1, 20.7. HRMS (ES) 702.4080 (MH$^+$). $C_{36}H_{56}N_5O_9$ requires 702.4078.

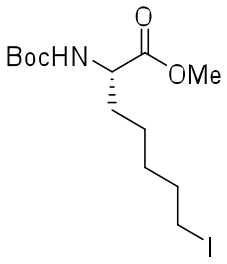
(*S*)-2-[(*S*)-2-Benzyloxycarbonylamino-3-(1H-imidazol-4-yl)-propionylamino]-4-methyl-pentanoic acid *tert*-butyl ester (4.22)



4.22

Cbz-Histidine **4.7** (10 g, 1 equiv) was coupled with leucine *tert*-butyl **4.20** (General procedure A1) and the crude product was purified by chromatography on silica gel and elution with methanol/dichloromethane (1:10) to give **4.22** as a white solid (14.1 g, 89%). ^{1}H NMR (CDCl$_3$, 500 MHz): δ 7.50 (1H, app d, *J* = 5.8Hz, N*H*), 7.43 (1H, s, 2-Ar*H*), 7.25 (5H, m, Ph), 6.74 (1H, s, 5-Ar*H*), 5.08 (2H, m, OC*H*$_2$Ar), 4.49 (1H, m, C*H*CH$_2$Ar), 4.34-4.37 (1H, m, C*H*CH$_2$CH(CH$_3$)$_2$), 3.08-3.12 (1H, dd, *J* = 4.2 and 14.5 Hz, CHC*H*HAr), 2.99-3.03 (1H, dd, *J* = 6.1 and 14.8 Hz, CHCH*H*Ar), 1.45-1.54 (3H, m, CHCH$_2$C*H*(CH$_3$)$_2$ and CHC*H*$_2$CH(CH$_3$)$_2$) 1.43 (9H, s, OC(C*H*$_3$)$_3$), 0.82-0.85 (6H, m, CHCH$_2$CH(C*H*$_3$)$_2$). ^{13}C NMR (CDCl$_3$, 75 MHz): δ 172.6, 171.5, 156.7, 136.4, 135.3, 128.7, 128.4, 128.2, 104.9, 82.3, 67.2, 54.8, 52.0, 41.2, 28.2, 24.9, 23.0, 22.0. HRMS (ES) 459.2600 (MH$^+$); $C_{24}H_{35}N_4O_5$ requires 459.2607.

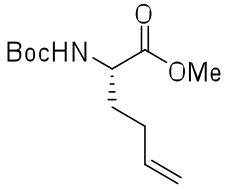
(*S*)-2-*tert*-Butoxycarbonylamino-7-iodo-heptanoic acid methyl ester (4.27).



4.27

Primary alcohol **4.33** (1.3 g, 4.7 mmol) was converted to the iodide **4.27** (General procedure L1). The crude product was purified by chromatography on silica gel and elution with ethyl acetate/petroleum ether (1:10) to give **4.27** as a light yellow oil (1.2 g, 66%). ^{1}H NMR ($CDCl_3$, 500 MHz): 5.02 (1H, app d, N*H*), 4.28 (1H, m, C*H*$CH_2CH_2CH_2CH_2CH_2$I), 3.74 (3H, s, OC*H*$_3$), 3.16-3.19 (2H, m, $CHCH_2CH_2CH_2CH_2CH_2$I), 1.79-1.83 (3H, m, CHC*H*$HCH_2CH_2CH_2CH_2$I and $CHCH_2CH_2CH_2CH_2CH_2$I), 1.61-1.64 (1H, $CHCH$*H*$CH_2CH_2CH_2CH_2$I), 1.44 (9H, s, (C*H*$_3$)$_3$), 1.35-1.42 (4H, m, $CHCH_2CH_2CH_2CH_2CH_2$I and $CHCH_2CH_2CH_2CH_2CH_2$I). ^{13}C NMR ($CDCl_3$, 75 MHz): 173.5, 155.5, 80.1, 53.5, 52.5, 33.4, 32.8, 30.2, 28.5, 24.5, 6.9. HRMS (ES) 386.0829 (MH^+). $C_{13}H_{25}NO_4$I requires 386.0828.

(*S*)-2-*tert*-Butoxycarbonylamino-hex-5-enoic acid methyl ester (4.29).

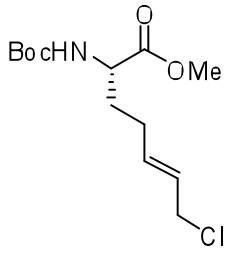


4.29

Carboxylic acid **4.28** (15 g, 65.4 mmol) was esterified with methyl iodide (General procedure J1) and the crude product purified by chromatography on silica gel and elution with ethyl acetate/petroleum ether (1:6) to give **4.29** as a colorless oil (11 g, 68%). ^{1}H NMR ($CDCl_3$, 500 MHz): 5.75-5.79 (1H, m, $CHCH_2CH_2CHCH_2$), 4.98-5.01 (2H, m, $CHCH_2CH_2CHCH_2$), 4.32 (1H, m, C*H*$CH_2CH_2CHCH_2$), 3.73 (3H, s, OC*H*$_3$), 2.10-2.12

(2H, m, $CHCH_2CH_2CHCH_2$)), 1.70-1.73 (1H, m, $CHCHHCH_2CHCH_2$), 1.65-1.67 (1H, m, , $CHCHHCH_2CHCH_2$), 1.43 (9H, m, $C(CH_3)_3$). ^{13}C NMR ($CDCl_3$, 75 MHz): 173.5, 155.5, 137.1, 115.9, 80.1, 53.2, 52.5, 32.2, 29.7, 28.5. HRMS (ES) 244.1547 (MH^+). $C_{12}H_{22}NO_4$ requires 244.1549.

(*E*)-(*S*)-2-*tert*-Butoxycarbonyloxy-7-chlorohept-5-enoic acid methyl ester (4.30).[3]



4.30

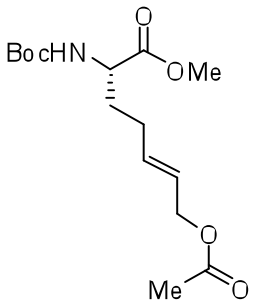
To a solution of (*S*)-2-*tert*-butoxycarbonyloxyhex-5-enoic acid methyl ester **4.29** (325 mg, 1.33 mmol) and allyl chloride (4 equiv) in dichloromethane (22 ml) was added Grubbs catalyst second generation (0.1 equiv). The reaction mixture was heated under reflux for 14 h and the solvent was evaporated under reduced pressure, The residue was chromatographed on silica gel and elution with ethyl acetate/petroleum ether (1:10) to give **4.30** (227 mg, 60%). ^1H NMR (500 MHz, $CDCl_3$) δ 5.70-5.73 (1H, dt, J = 15.1 and 6.6 Hz), 5.64-5.66 (1H, dt, J = 15.1, 6.6 Hz), 5.02 (1H, app d, J = 8.0 Hz, N*H*), 4.28 (1H, m, $CHCH_2CH_2CHCH$), 3.99 (2 H, d, J = 6.6 Hz, $CHCHCH_2Cl$), 3.70 (3H, s, OCH_3), 2.07-2.12 (2 H, m, $CHCH_2CH_2CHCH$), 1.84-1.88 (1H, m, $CHCHHCH_2CHCH$), 1.64-1.69 (1H, m, $CHCHHCH_2CHCH$), 1.40 (9 H, s, OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.3, 155.5, 134.1, 127.4, 80.2, 53.1, 52.6, 45.2, 32.2, 28.5, 28.1. MS (ES) 292.13 (MH^+). $C_{13}H_{23}ClNO_4$ requires 292.13.

Lit cit: [3]Eustache, J.; Weghe, P. V. D.; Nouen, D. L.; Uyehara, H.; Kabuto, C.; Yamamoto, Y. *J. Org. Chem.,* **2005**, *70*, 4043-4053.

^1H NMR (400 MHz, $CDCl_3$) δ 1.44 (9 H, s), 1.66-1.77 (1 H, m), 1.96 (1 H, m), 2.09-2.20 (2 H, m), 3.74 (3 H, s), 4.02 (2 H, d, J = 6.6 Hz), 4.26-4.35 (1 H, m), 4.96-5.08 (1 H, m), 5.65 (1H, dt, J = 15.1, 6.6 Hz), 5.75 (1H, dt, J = 15.1, 6.6 Hz); ^{13}C NMR (100 MHz,

CDCl3) δ 27.9, 28.4, 32.1, 45.1, 52.4, 53.0, 80.0, 127.2, 133.8, 161.6, 173.0; HRMS (ESI, positive mode) calcd for $C_{13}H_{22}ClNO_4$ + Na 314.1135, found 314.1130.

(*E*)-(*S*)-7-Acetoxy-2-*tert*-butoxycarbonyloxyhept-5-enoic acid methyl ester (4.31).[3]



4.31

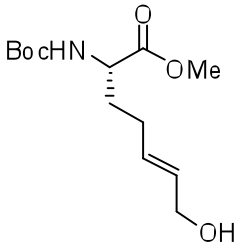
A mixture of allylic chloride **4.30** (560 mg, 1.92 mmol) and NaOAc (156 mg, 10 equiv) in DMF (10 ml), was heated at 100 °C for 23 h. Additional NaOAc (50 mg) was added and stirring continued for 3 h. The reaction mixture was cooled to rt, diluted with ether, and washed with saturated NH_4Cl. The organic layer was dried and concentrated under reduced pressure. The residue was purified by chromatography on silica gel and elution with ethyl acetate/petroleum ether (1:4) to give **4.31** (409 mg, 68%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.71-5.74 (1H, dt, *J* = 15.1 and 6.6 Hz), 5.57-5.61 (1H, dt, *J* = 15.1, 6.6 Hz), 5.03 (1H, d, *J* = 8.0 Hz, N*H*), 4.48 (2 H, d, *J* = 6.6 Hz, CHCHCH_2OCOCH$_3$), 4.29 (1H, m, C*H*CH$_2$CH$_2$CHCH), 3.72 (3H, s, OCH_3), 2.11-2.14 (2 H, m, CHCH$_2$CH_2CHCH), 2.04 (3 H, m, CHCHCH$_2$OCOCH_3), 1.88-1.91 (1H, m, CHC*H*HCH$_2$CHCH), 1.68-1.72 (1H, m, CHCH*H*CH$_2$CHCH), 1.42 (9 H, s, OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.4, 17.0, 155.5, 134.4, 128.6, 80.2, 65.1, 53.2, 52.5, 32.2, 28.5, 28.2, 21.2. MS (ES) 338.15 (MNa^+). $C_{15}H_{25}NNaO_6$ requires 338.15.

Lit cit: [3]Eustache, J.; Weghe, P. V. D.; Nouen, D. L.; Uyehara, H.; Kabuto, C.; Yamamoto, Y. *J. Org. Chem.,* **2005**, *70*, 4043-4053.

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43 (9 H, s), 1.66-1.77 (1 H, m), 1.86-1.97 (1 H, m), 2.06 (3 H, m), 2.08-2.23 (2 H, m), 3.74 (3 H, s), 4.25-4.36 (1H, m), 4.50 (2 H, d, *J* = 6.3 Hz), 4.99-5.10 (1 H, m), 5.55-5.69 (1 H, m), 5.74 (1H, dt, *J* = 15.3, 6.6 Hz); ^{13}C NMR (100

MHz, $CDCl_3$) δ 21.2, 28.1, 28.4, 32.1, 52.3 53.0, 64.9, 80.0, 125.1, 134.0, 155.2, 170.6, 172.9; HRMS (ESI, positive mode) calcd for $C_{15}H_{25}NO_6$ + Na 338.1580, found 338.1574.

(*E*)-(*S*)-2-*tert*-Butoxycarbonyloxy-7-hydroxyhept-5-enoic acid methyl ester (4.32).[3]



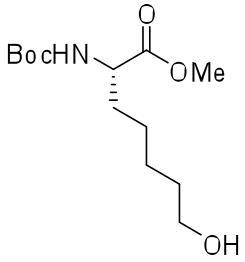
4.32

The acetate **4.31** (400 mg, 1.27 mmol) was dissolved in a solution of NaOMe in methanol (0.1 M). The mixture was stirred for 2.5 h, and aqueous HCl (1 N, 4 ml) was added, followed by ether. The organic layer was washed with H_2O and brine and dried over $MgSO_4$, and the solvent removed under reduced pressure to give allylic alcohol **4.32** as an oil which was not further purified (298 mg, 86%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.59-5.65 (2H, m, C*H*C*H*CH_2OH), 5.09 (1H, d, *J* = 8.1 Hz, NH), 4.25 (1H, m, C*H*CH_2CH_2CHCH), 4.04 (2H, m, CHCHC*H*$_2$OH), 3.69 (3H, s, OC*H*$_3$), 2.05-2.08 (2H, m, $CHCH_2$C*H*$_2$CHCH), 1.83-1.88 (1H, m, CHC*H*HC*H*$_2$CHCH), 1.62-1.70 (1H, m, CHCH*H*C*H*$_2$CHCH), 1.39 (9H, s, C(C*H*$_3$)$_3$). MS (ES) 274.1 (MH^+). $C_{13}H_{24}NO_5$ requires 274.1.

Lit cit: [3]Eustache, J.; Weghe, P. V. D.; Nouen, D. L.; Uyehara, H.; Kabuto, C.; Yamamoto, Y. *J. Org. Chem.,* **2005**, *70*, 4043-4053.

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 (9 H, s), 1.65-1.77 (1 H, m), 1.85-1.97 (1 H, m), 2.09-2.19 (2 H, m), 3.74 (3 H, s), 4.08 (2 H, t, J = 4.1 Hz), 4.27-4.37 (1 H, m), 4.95-5.03 (1 H, m), 5.72-5.60 (2 H, m); 13C NMR (100 MHz, $CDCl_3$) δ 28.0, 28.4, 32.1, 52.3, 52.8, 63.4, 80.0, 130.5, 130.7, 155.2, 173.2; HRMS (ESI, positive mode) calcd for $C_{13}H_{23}NO_5$ + Na 296.1474, found 296.1468.

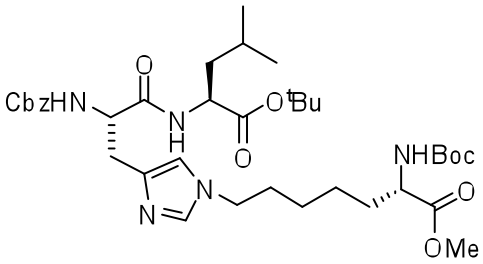
(*S*)-2-*tert*-Butoxycarbonylamino-7-hydroxy-heptanoic acid methyl ester (4.33).



4.33

The allylic alcohol **4.32** (298 mg, 1.08 mmol) was hydrogenated in a hydrogen atmosphere over platinum oxide (30 mg, 10 mol% w/w) in ethyl acetate for 16 hours. The mixture was filtered and solution was concentrated *in vacuo*. The crude product was purified by silica gel column chromatography and elution with ethyl acetate/petroleum ether (1:2) to give **4.33** (280 mg, 94%) as a thick oil. ^{1}H NMR ($CDCl_3$, 500 MHz): 5.01 (1H, d, J = 5.3 Hz, N*H*), 4.3 (1H, m, C*H*$CH_2CH_2CH_2CH_2CH_2OH$), 3.74 (3H, s, OCH_3), 3.62-3.65 (2H, m, $CHCH_2CH_2CH_2CH_2CH_2OH$), 1.79-1.81 (1H, m, CHC*H*$HCH_2CH_2CH_2CH_2OH$), 1.59-1.63 (1H, m, $CHCHHCH_2CH_2CH_2CH_2OH$), 1.57-1.59 (2H, m, $CHCH_2CH_2CH_2CH_2CH_2OH$), 1.45 (9H, s, C(CH_3)$_3$), 1.37-1.40 (4H, $CHCH_2CH_2CH_2CH_2CH_2OH$ and $CHCH_2CH_2CH_2CH_2CH_2OH$). ^{13}C NMR ($CDCl_3$, 75 MHz): 173.8, 155.3, 80.1, 62.6, 53.6, 52.4, 33.0, 32.9, 28.5, 25.1, 22.6. HRMS (ES) 276.1814 (MH^+). $C_{13}H_{26}NO_5$ requires 276.1811.

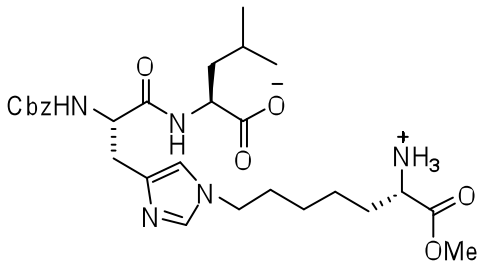
7-{[(*E*)-(*S*)-4-Benzyloxycarbonylamino-4-((*S*)-1-*tert*-butoxycarbonyl-3-methyl-butyl carbamoyl)-2-methyl-but-1-enyl]-vinyl-amino}-2-*tert*-butoxycarbonylamino-heptanoic acid methyl ester (4.35).



4.35

Alkylation of *N*-Cbz-His-O^{t}Bu **4.22** (488 mg, 1.06 mmol) with alkyl iodide **4.27** (General procedure M2) gave the crude product that was purified by column chromatography on silica gel by elution with ethyl acetate/petroleum ether (6:1) to give gave **4.35** as a yellow oil (430 mg, 56%). ^{1}H NMR (CD_3OD, 500 MHz): 7.88 (1H, s, 2-Ar*H*), 7.29-7.33 (5H, m, Ph), 7.03 (1H, s, 5-Ar*H*), 6.95 (1H, d, *J*= 8.3 Hz, N*H*), 5.05 (2H, m, OCH_2Ar), 4.40-4.43 (1H, m, C*H*CH_2Ar), 4.30-4.33 (1H, m, C*H*CH_2CH(CH_3)$_2$), 4.06-4.10 (1H, m, C*H*CH_2CH_2CH_2CH_2CH_2Ar), 3.95-3.98 (2H, m, CHCH_2CH_2CH_2CH_2CH_2Ar), 3.69 (3H, s, OCH_3), 3.06-3.09 (1H, dd, *J* = 5.3 and 14.8 Hz, CHC*H*HAr), 2.86-2.89 (1H, dd, *J* = 8.4 and 14.4 Hz, CHCH*H*Ar), 1.74-1.78 (5H, m, CHCH_2CH_2CH_2CH_2CH_2Ar, CHCH_2C*H*(CH_3)$_2$ and CHCH_2CH(CH_3)$_2$), 1.43-1.61 (4H, m, CHCH_2CH_2CH_2CH_2CH_2Ar and CHCH_2CH_2CH_2CH_2CH_2Ar). 1.45 (9H, s, OC(CH_3)$_3$), 1.43 (9H, s, OC(CH_3)$_3$), 1.28-1.38 (2H, m, CHCH_2CH_2CH_2CH_2CH_2Ar), 0.95 (3H, d, *J* = 6.6 Hz, CH_3), 0.89 (3H, d, *J* = 6.4 Hz, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): 173.8, 172.4, 172.1, 156.9, 137.0, 136.1, 128.3, 127.9, 127.7, 118.1, 81.5, 79.4, 66.4, 54.7, 53.7, 51.8, 51.4, 46.6, 40.3, 31.3, 30.3, 27.5, 27.1, 25.7, 25.2, 24.8, 22.1, 20.7. HRMS (ES) 716.4239 (MH^+); $C_{37}H_{58}N_5O_9$ requires 716.4235.

(*S*)-2-(2-Benzyloxycarbonylamino-acetylamino)-4-methyl-pentanoate1-methoxy carbonyl -6-(4-methyl-imidazol-1-yl)-hexyl-ammonium (4.36)

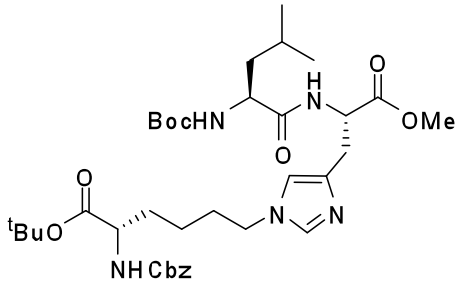


4.36

Acidolysis of pseudo-tripeptide **4.35** (430 mg, 0.6 mmol) in the presence of trifluoroacetic acid (General procedure N) afforded **4.36** as a thick oil (99%).^{1}H NMR for major product from mixture (CD_3OD, 500 MHz): 8.80 (1H, s, 2-Ar*H*), 8.37 (1H, d , *J* = 7.7 Hz, N*H*), 7.38 (1H, s, 5-Ar*H*), 7.31-7.36 (5H, m, Ph), 5.07 (2H, m, OCH_2Ar), 4.48-4.51 (1H, m, C*H*CH_2Ar), 4.41-4.43 (1H, m, C*H*CH_2CH(CH_3)$_2$), 4.13-4.16 (2H, m,

CHCH$_2$CH$_2$CH$_2$CH$_2$C*H*$_2$Ar), 4.02-4.04 (1H, m, C*H*CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$Ar), 3.82 (3H, s, OCH$_3$), 3.21-3.25 (1H, dd, *J* = 6.4 and 15.4 Hz, CHC*H*HAr), 3.03-3.07 (1H, dd, *J* = 7.4 and 15.2 Hz, CHCH*H*Ar), 1.83-1.96 (4H, CHC*H*$_2$CH$_2$CH$_2$CH$_2$CH$_2$Ar and CHCH$_2$CH$_2$CH$_2$C*H*$_2$CH$_2$Ar), 1.63-1.72 (3H, m, CHCH$_2$C*H*(CH$_3$)$_2$ and CHC*H*$_2$CH(CH$_3$)$_2$), 1.34-1.52 (4H, m, CHCH$_2$CH$_2$C*H*$_2$CH$_2$CH$_2$Ar and CHCH$_2$C*H*$_2$CH$_2$CH$_2$CH$_2$Ar), 0.94 (3H, d, *J* = 6.4 Hz, CHC*H*$_2$CH(C*H*$_3$)$_2$), 0.90 (3H, d, *J* = 6.1 Hz, CHC*H*$_2$CH(C*H*$_3$)$_2$). ^{13}C NMR (CD$_3$OD, 75 MHz): 174.8, 171.1, 169.9, 156.8, 136.9, 134.5, 130.3, 128.4, 128.0, 127.8, 120.2, 66.7, 53.9, 52.6, 52.5, 49.2, 44.1, 40.1, 30.1, 29.4, 27.5, 25.5, 24.8, 24.2, 22.2, 20.5. HRMS (ES) 560.3077 (MH$^+$); C$_{28}$H$_{42}$N$_5$O$_7$ requires 560.3084.

(*S*)-2-Benzyloxycarbonylamino-6-{4-[(*S*)-2-((*S*)-2-*tert*-butoxycarbonylamino-4-methyl pentanoylamino)-2-methoxycarbonyl-ethyl]-imidazol-1-yl}-hexanoic acid *tert*-butyl ester (4.39).

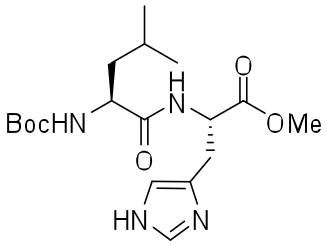


4.39

Alkylation of *N*-Boc-Leu-His-OMe **4.40** (1.14 g, 2.98 mmol) with alkyl iodide **4.41** (General procedure M2) gave the crude material. The crude product was purified by chromatography on silica gel and elution with methanol/dichloromethane (1:20) to give **4.39** as a yellow oil (860 mg, 41%). ^{1}H NMR (CD$_3$OD, 500 MHz): 7.52 (1H, s, 2-Ar*H*), 7.32-7.37 (5H, m, Ph), 6.93 (1H, s, 5-Ar*H*), 5.09 (2H, m, OC*H*$_2$Ar), 4.85 (1H, m, C*H*CH$_2$Ar), 4.07 (1H, m,C*H*CH$_2$CH(CH$_3$)$_2$), 4.01 (1H, m, C*H*CH$_2$CH$_2$CH$_2$ CH$_2$Ar), 3.92-3.95 (2H, m, CHCH$_2$CH$_2$CH$_2$C*H*$_2$Ar), 3.67 (3H, s, OC*H*$_3$), 3.03-3.07 ((1H, dd, *J* = 4.9 and 14.8 Hz, CHC*H*HAr) 2.82-2.85 (1H, m, CHCH*H*Ar), 1.74-1.80 (3H, m, CHCH$_2$CH$_2$C*H*$_2$CH$_2$N and CHC*H*HCH$_2$CH$_2$ CH$_2$N), 1.63-1.69 (2H, m, CHCH*H*CH$_2$CH$_2$CH$_2$N and CHCH$_2$C*H*(CH$_3$)$_2$), 1.45-1.50 (2H, m, CHC*H*$_2$CH (CH$_3$)$_2$), 1.43 (9H, s, OC(C*H*$_3$)$_3$), 1.42 (9H, s, OC(C*H*$_3$)$_3$), 1.32-1.39 (2H, m,

$CHCH_2CH_2CH_2CH_2N$), 0.91-0.95 (6H, m, $CHCH_2CH(CH_3)_2$). ^{13}C NMR (CD_3OD, 126 MHz): 174.4, 172.1, 171.9, 157.4, 156.5, 137.0, 136.7, 128.3, 127.8, 127.7, 117.2, 81.5, 79.3, 66.4, 54.8, 53.2, 52.6, 51.5, 46.6, 40.9, 31.0, 30.3, 29.8, 27.6, 27.0, 24.6, 22.6, 22.3, 20.7. HRMS (ES) 702.4044 (MH^+); $C_{36}H_{56}N_5O_9$ requires 702.4078.

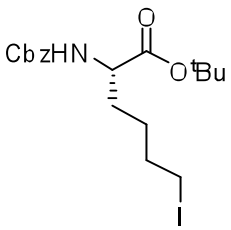
(*Z*)-(*S*)-2-((*S*)-2-*tert*-Butoxycarbonylamino-4-methyl-pentanoylamino)-5-methyl amino-4-methyleneamino-pent-4-enoic acid methyl ester (4.40).



4.40

Histidine methyl ester **4.49** (10.7 g, 44.5 mmol) was coupled with Boc-Leucine **4.50** (General procedure A1) and the crude product was purified by chromatography on silica gel and elution with methanol/dichloromethane (1:10) to give **4.40** as a white solid (14.4 g, 85%). ^1H NMR ($CDCl_3$, 500Hz): δ 7.48 (1H, s, 2-Ar*H*), 7.36 (1H, appr s, N*H*), 6.71 (1H, s, 5-Ar*H*), 5.26 (1H, d, J = 7.2 Hz, N*H*), 4.72-4.76 (1H, m, C*H*CH_2Ar), 4.04-4.05 (1H, m, C*H*$CH_2CH(CH_3)_2$), 3.65 (3H, s, OCH_3), 3.07-3.10 (2H, m, CHC*H*$_2$Ar), 1.61-1.67 (1H, m, $CHCH_2CH(CH_3)_2$), 1.53-1.60 (1H, m, CHC*H*$HCH(CH_3)_2$), 1.41-1.48 (1H, m, $CHCHHCH(CH_3)_2$), 1.38 (9H, s, $C(CH_3)_3$), 0.86-0.89 (6H, m, $CHCH_2CH(CH_3)_2$). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 173.2, 171.6, 156.3, 135.6, 130.7, 120.0, 80.4, 53.7, 52.9, 52.6, 41.4, 28.5, 24.8, 23.2, 23.0, 22.2. HRMS (ES) 383.2279 (MH^+); $C_{18}H_{31}N_4O_5$ requires 383.2294.

(*S*)-2-Benzyloxycarbonylamino-6-iodo-hexanoic acid *tert*-butyl ester (4.41).[4]



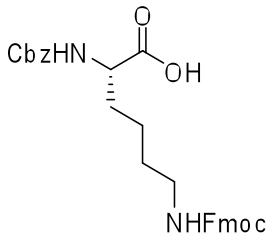
4.41

Primary alcohol **4.46** (5.9 g, 17.5 mmol) was converted to the iodide **4.41** (General procedure L1). The crude product was purified by chromatography on silica gel and elution with ethyl acetate/petroleum ether (1:10) to give **4.41** as a light yellow oil (4.86 g, 62%). ^{1}H NMR ($CDCl_3$): δ 7.34-7.36 (5H, m, Ph), 5.37 (1H, d, *J* = 6.7, N*H*), 5.10 (2H, s, OC*H*$_2$Ph), 4.25 (1H, m, C*H*CH$_2$CH$_2$CH$_2$CH$_2$I), 3.16 (2H, m, CHCH$_2$CH$_2$CH$_2$C*H*$_2$I), 1.79-1.85 (2H, m, CHCH$_2$CH$_2$C*H*$_2$CH$_2$I and CHC*H*HCH$_2$CH$_2$CH$_2$I), 1.64 (1H, m, CHCH*H*CH$_2$CH$_2$CH$_2$I), 1.44-1.47 (2H, m, CHCH$_2$C*H*$_2$CH$_2$CH$_2$I), 1.45 (9H, s, C(C*H*$_3$)$_3$). HRMS (ES) 448.0975 (MH$^+$); $C_{18}H_{27}NO_4I$ requires 448.0985.

Lit cit: [4]Allevi, P.; Galligani, M.; Anastasia, M. *Tetrahedron: Asymm.,* **2002**, *13*, 1901–1910.

^{1}H NMR ($CDCl_3$): 7.40–7.28 (5H, aromatics-H), 5.33 (1H, d, *J* = 7.4, N*H*), 5.11–5.05 (2H, AB system, OC*H*$_2$Ph), 4.23 (1H, m, 2-H), 3.14 (2H, t, *J* = 6.7, 6-H$_2$), 1.87–1.75 (3H, overlapping), 1.64 (1H, m), 1.48–1.40 (2H, overlapping), 1.45 [9H, s, C(C*H*$_3$)$_3$]. Anal. calcd for $C_{18}H_{26}INO_4$: C, 48.33; H, 5.86; N, 3.13. Found: C, 48.25; H, 5.88; N, 3.23%.

(*S*)-2-Benzyloxycarbonylamino-6-(9H-fluoren-9-ylmethoxycarbonylamino)-hexanoic acid (4.43).[5]



4.43

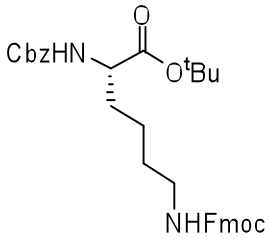
Cbz-Lys-OH **4.42** (2.8 g, 10.6 mmol) was allowed to react with Fmoc-Cl (General procedure Q). to give **4.43** (5.119 g, 92%) without purification. ^{1}H NMR (CD_3OD, 500 MHz): 7.77 (2H, d, *J* = 7.3Hz, Ar*H*), 7.62 (2H, d, *J* = 7.5Hz, Ar*H*), 7.23-7.38 (9H, m, Ar*H*), 5.05 (2H, m, ArC*H*$_2$), 4.30 (2H, m, ArC*H*$_2$OCONH), 4.17 (1H, t, *J* = 6.8Hz, Fmoc*H*), 4.04 (1H, m, C*H*CH$_2$CH$_2$CH$_2$CH$_2$NH), 3.08 (2H, m, CHCH$_2$CH$_2$CH$_2$C*H*$_2$NH), 1.81-1.84 (1H, m, CHC*H*HCH$_2$CH$_2$CH$_2$NH), 1.64-1.68 (1H, m, CHCH*H*CH$_2$CH$_2$

CH_2NH), 1.44-1.51 (2H, m, $CHCH_2CH_2CH_2CH_2NH$), 1.37-1.41 (2H, m, $CHCH_2CH_2CH_2CH_2NH$). ^{13}C NMR (CD_3OD, 126 MHz): 178.1, 157.7, 157.1, 144.1, 141.4, 137.1, 128.2, 127.7, 127.6, 127.5, 126.9, 125.0, 119.7, 66.4, 66.2, 56.2, 47.3, 40.3, 32.4, 29.4, 22.8. HRMS (ES) 525.1999 (MNa^+); $C_{29}H_{30}N_2NaO_6$ requires 525.1996.

Lit cit: [5]Marrano, C.; Mace´do, P.; Gagnon, P.; Lapierre, D.; Gravel, C.; Keillor, J. W. *Bioorganic & Medicinal Chemistry*, **2001**, *9*, 3231–3241.

1H NMR($CDCl_3$, 400 MHz) δ 7.75 (d, 2H, J = 7.5 Hz), 7.56 (d, 2H, J = 7.4 Hz), 7.43–7.28 (m, 9H), 6.43 (s, 1H), 5.61 (s, 1H), 5.10 (s, 2H), 4.44–4.36 (m, 3H), 4.18 (t, 1H, J = 6.5 Hz), 3.18 (t, 2H, J = 4.6 Hz), 1.54–1.24 (m, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 175.95, 156.97, 156.58, 143.98, 141.33, 136.28, 128.57, 128.21, 128.13, 127.15, 125.15, 125.01, 120.06, 67.09, 66.69, 53.76, 47.24, 40.56, 31.78, 29.26, 22.41. MS (FAB+) 503.4 (MH+). Mp 139 °C.

(*S*)-2-Benzyloxycarbonylamino-6-(9H-fluoren-9-ylmethoxycarbonylamino)-hexanoic acid *tert*-butyl ester (4.44)



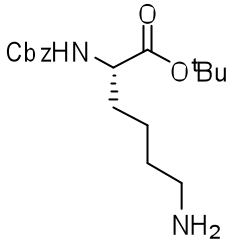
4.44

DCC mediated esterification of carboxylic acid **4.43** (1.1 g, 2.18 mmol) with *tert*-butyl alcohol (General procedure K1) gave the ester. The crude product was purified by chromatograghy on silica gel and elution with ethyl acetate/petroleum ether (1:3) to give **4.44** as a white solid (548 mg, 45%).

EDCI mediated esterification of carboxylic acid **4.43** (500 mg, 1 mmol) with *tert*-butyl alcohol (General procedure K2) gave the ester. The crude product was purified by chromatography on silica gel and elution with ethyl acetate/petroleum ether (1:3) to give **4.44** as a white solid (363 mg, 65%). ^1H NMR($CDCl_3$, 500 MHz): 7.75 (2H, d, J=7.5 Hz),

7.58 (2H, d, *J* = 7.4 Hz), 7.26-7.41 (9H, m, Ar*H*), 5.34 (1H, NH, d, *J* = 7.9Hz), 5.10 (2H, s, ArCH_2), 4.85 (1H, app s, N*H*), 4.38-4.40 (2H, ArHCH_2), 4.22 (1H, C*H*CH_2 $CH_2CH_2CH_2NH$), 4.20 (1H, Ar*H*CH_2), 3.18 (2H, m, $CHCH_2CH_2CH_2CH_2NH$), 1.82 (1H, m, CHC*H*$HCH_2CH_2CH_2NH$), 1.66 (1H, m, $CHCHHCH_2CH_2CH_2NH$), 1.52-1.55 (2H, m, $CHCH_2CH_2CH_2CH_2NH$), 1.46 (9H, s, $C(CH_3)_3$), 1.33-1.42 (2H, overlapping, CH $CH_2CH_2CH_2CH_2NH$). ^{13}C NMR (CD_3OD, 75 MHz): 171.7, 156.7, 156.2, 144.2, 141.5, 128.7, 128.4, 128.3, 127.9, 127.3, 125.3, 120.2, 82.4, 67.1, 66.8, 54.3, 47.5, 40.9, 32.8, 29.7, 28.2, 22.4. HRMS (ES) 559.2808 (MH^+); $C_{33}H_{39}N_2O_6$ requires 559.2808.

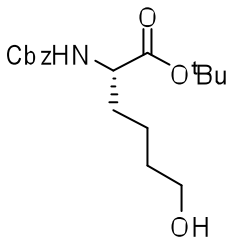
(*S*)-6-Amino-2-benzyloxycarbonylamino-hexanoic acid *tert*-butyl ester (4.45).



4.45

Fmoc protecting group of **4.44** (1.2 g, 2.15 mmol) was removed on treatment with diethylamine (General procedure R) to give **4.45** (652 mg, 90%). ^{1}H NMR ($CDCl_3$): δ 7.31-7.36 (5H, m, Ph), 5.34 (1H, d, *J* = 8.0 Hz, N*H*), 5.12 (2H, s, OCH_2Ph), 4.25 (1H, m, C*H*$CH_2CH_2CH_2CH_2NH_2$), 2.64-2.67 (2H, m, $CHCH_2CH_2CH_2CH_2NH_2$), 1.74-1.83 (2H, m, $CHCH_2CH_2CH_2CH_2NH_2$), 1.63-1.68 (4H, m, $CHCH_2CH_2CH_2CH_2NH_2$ and $CHCH_2CH_2CH_2CH_2NH_2$), 1.45 (9H, s, $C(CH_3)_3$). MS (ES) 337.2 (MH^+); $C_{18}H_{29}N_2O_4$ requires 337.2.

(*S*)-2-Benzyloxycarbonylamino-6-hydroxy-hexanoic acid *tert*-butyl ester (4.46).[4]



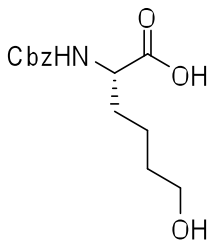
4.46

Diazotization of lysine derivative **4.45** (3.1 g, 9.2 mmol) (General procedure I2) gave the crude product that was purified by flash column chromatography on silica gel and elution with ethyl acetate/petroleum ether (1:1) to give **4.46** as a white solid (1.31 g, 42%).[1]H NMR ($CDCl_3$): δ 7.30-7.36 (5H, m, Ph), 5.36 (1H, d, *J* = 6.7 Hz, N*H*), 5.11 (2H, s, OC*H*$_2$Ph), 4.25 (1H, m, C*H*$CH_2CH_2CH_2CH_2OH$), 3.62 (2H, m, $CHCH_2CH_2CH_2CH_2OH$), 1.80-1.85 (2H, m, $CHCH_2CH_2CH_2CH_2CH_2OH$), 1.58-1.72 (4H, m, $CHCH_2CH_2CH_2CH_2OH$ and $CHCH_2CH_2CH_2CH_2OH$), 1.45 (9H, s, $C(CH_3)_3$). ^{13}C NMR ($CDCl_3$, 126 MHz): 171.5, 156.1, 136.3, 127.9, 127.2, 125.2, 82.3, 67.3, 60.6, 53.2, 31.9, 31.1, 29.5, 21.3. HRMS (ES) 338.1953 (MH^+); $C_{18}H_{28}NO_5$ requires 338.1967.

Lit cit: [4]Allevi, P.; Galligani, M.; Anastasia, M. *Tetrahedron: Asymm.,* **2002**, *13*, 1901–1910.

[1]H NMR ($CDCl_3$): 7.33–7.26 (5H, aromatics-H), 5.35 (1H, d, J = 7.5, NH), 5.07 (2H, s, OCH_2Ph), 4.22 (1H, m, 2-H), 3.58 (2H, t, J=6.1, 6-H_2), 1.78 (1H, m), 1.63 (1H, m), 1.54 (2H, m), 1.43 [9H, s, $C(CH_3)_3$] 1.41–1.36 (2H, overlapping). Anal. calcd for $C_{18}H_{27}NO_5$: C, 64.07; H, 8.07; N, 4.15. Found: C, 64.13; H, 8.00; N, 4.13%.

(*S*)-2-Benzyloxycarbonylamino-6-hydroxy-hexanoic acid (4.48).



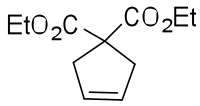
4.48

Diazotization of Cbz-Lysine **4.42** (40 g, 143 mmol) (General procedure I1) gave crude product that was purified by flash column chromatography on silica gel and elution with ethyl acetate/petroleum ether (2:1) to give **4.48** as an oil (18.1 g, 45%). [1]H NMR (CD_3OD, 500 MHz): 7.28-7.35 (5H, m, Ph), 5.09 (2H, m, PhC*H*$_2$), 4.14-4.17 (1H, C*H*$CH_2CH_2CH_2$ CH_2OH), 3.54 (2H, m, $CHCH_2CH_2CH_2CH_2OH$), 1.83-1.87 (1H, m, CHC*H*$HCH_2CH_2CH_2OH$), 1.66-1.71 (1H, m, $CHCHHCH_2CH_2CH_2OH$), 1.51-1.57 (2H, m, $CHCH_2CH_2CH_2CH_2OH$), 1.43-1.50 (2H, m, $CHCH_2CH_2CH_2CH_2OH$) ^{13}C NMR

(CD_3OH, 126 MHz): 174.8, 157.5, 137.0, 128.2, 127.8, 127.6, 66.4, 61.4, 54.1, 31.9, 31.3, 22.1. HRMS (ES) 282.1332 (MH^+); $C_{14}H_{20}NO_5$ requires 282.1341.

7.5: Experimental work described in chapter 5

Cyclopent-3-ene-1,1-dicarboxylic acid diethyl ester (5.29)



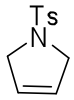
5.29

The diene **5.28** (28 mg) was subjected to RCM (General procedure C6). Conversion to **5.29** was determined by analysis of the ^{1}H NMR spectrum of the crude product. Data for **5.29** (from mixture): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.62 (m, 2H), 4.18 (m, 4H), 2.62 (m, 4H), 1.22 (m, 6H).

Lit cit: Yao, Q. *J. Am. Chem. Soc.* **2004**, *126*, 74–75.

H NMR ($CDCl_3$, 500 MHz): δ 5.62 (s, 2H), 4.21 (q, 4H, J = 7.2 Hz), 3.02 (s, 4H), 1.26 (t, 6 H, J = 7.1 Hz)

1-(Toluene-4-sulfonyl)-2,5-dihydro-1H-pyrrole (5.31)



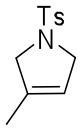
5.31

The diene **5.30** (15.8 mg) was subjected to RCM (General procedure C6). Conversion to **5.31**was determined by analysis of the ^{1}H NMR spectrum of the crude product. Data for **5.31** (from mixture): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.70 (m, 2H), 7.29 (m, 2H), 5.61 (s, 2H), 4.08 (s, 4H), 2.39 (s, 3H).

Lit cit: *Varray, S; Organometallics* , **2003**, *22*, 2426

^{1}H NMR ($CDCl_3$, Me_4Si) δ 2.45 (s, 3 H), 4.10 (s, 4 H), 5.65 (s, 2 H), 7.30 (d, J = 8.5 Hz, 2 H), 7.75 (d, J = 8.5 Hz, 2H); MS (electrospray) m/z 224 (M+ H)+.

3-Methyl-1-(toluene-4-sulfonyl)-2,5-dihydro-1H-pyrrole (5.33)



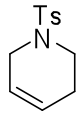
5.33

The diene **5.32** (36 mg) was subjected to RCM (General procedure C6) to give **5.33**. Conversion to **5.33** was determined by analysis of the ^{1}H NMR spectrum of the crude product. Data for **5.33** (from mixture): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.72 (m, 2H), 7.26 (m, 2H), 5.25 (m, 1H), 4.07 (m, 2H), 3.96 (m, 2H), 2.42 (s, 3H), 1.65 (s, 3H).

Lit cit: Yao, Q. *J. Am. Chem. Soc.* **2004**, *126*, 74–75.

^{1}H NMR ($CDCl_3$, 500 MHz): 7.72 (d, 2 H, J = 8.2 Hz), 7.32 (d, 2 H, J = 8.1 Hz), 5.25 (m, 1 H), 4.06-4.08 (m, 2 H), 3.97 (bs, 2 H), 2.43 (s, 3 H), 1.66 (s, 3 H).

1-(Toluene-4-sulfonyl)-1,2,3,6-tetrahydro-pyridine (5.35)



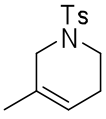
5.35

The diene **5.34** (31.8 mg) was subjected to RCM (General procedure C6). Conversion to **5.35** was determined by analysis of the ^{1}H NMR spectrum of the crude product. Data for **5.35** (from mixture): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.69 (m, 2H), 7.31 (m, 2H), 5.76 (m, 1H), 5.61 (m, 1H), 3.64 (m, 2H), 3.38 (m, 2H), 2.43 (m, 3H), 2.21 (m, 2H);

Lit cit: Lipshutz, B. H.; Ghorai, S.; Abela, A. R.; Moser, R.; Nishikata, T.; Duplais, C.; Krasovskiy, A. *J. Org. Chem.* **2011**, *76*, 4379–4391.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, J = 8.0 Hz, 2H), 7.32 (d, J = 8.0 Hz, 2H), 5.77-5.72 (m, 1H), 5.63-5.58 (m, 1H), 3.58-3.55 (m, 2H), 3.16 (t, J = 6.0 Hz, 2H), 2.42 (s, 3H), 2.24-2.18 (m, 2H).

5-Methyl-1-(toluene-4-sulfonyl)-1,2,3,6-tetrahydro-pyridine (5.37)



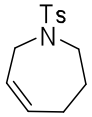
5.37

The diene **5.36** (33.5 mg) was subjected to RCM (General procedure C6). Conversion to **5.37** was determined by analysis of the ^{1}H NMR spectrum of the crude product.Data for **5.37** (from mixture), ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.70 (m, 2H), 7.31 (m, 2H), 5.42 (m, 2H), 3.70 (m, 2H), 3.15 (m, 2H), 2.42 (m, 3H), 2.21 (m, 2H), 1.71 (m, 3H).

Lit cit: Tamaru, Y.; Hojo, M.; Yoshida, Z.-i. *J. Org. Chem*. **1988**, *53*, 5731–5741.

^{1}H NMR (CDCl,) δ 1.65 (br s, 3 H), 2.10 (m, 2 H), 2.43 (5, 3 H), 3.16 (t, J = 5.8 Hz, 2 H), 3.52 (m, 2 H), 5.29 (m, 1 H); ^{13}C NMR ($CDC1_3$) 6 21.2, 22.6, 30.0, 42.7,44.6,116.7,127.6, 129.4, 134.4, 143.1.

1-(Toluene-4-sulfonyl)-2,3,4,7-tetrahydro-1H-azepine (5.39)



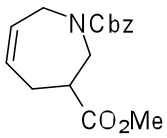
5.39

The diene **5.38** (33.5 mg) was subjected to RCM (General procedure C6). Conversion to **5.39** was determined by analysis of the ^{1}H NMR spectrum of the crude product. Data for **5.39** (from mixture): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.69 (m, 2H), 7.29 (m, 2H), 5.74 (m, 1H), 5.61 (m, 1H), 3.79 (m, 2H), 3.12 (m, 2H), 2.41 (m, 3H), 2.02 (m, 2H), 1.62 (m, 2H). MS (ES) 252.10 (MH^+); $C_{13}H_{18}NO_2S$ requires: 252.10.

Lit cit: Yao, Q. *J. Am. Chem. Soc.* **2004**, *126*, 74–75.

^{1}H NMR ($CDCl_3$, 500 MHz): 7.69 (d, 2 H, *J* = 8.2 Hz), 7.29 (d, 2 H, *J* = 8.1 Hz), 5.76-5.80 (m, 1 H), 5.64-5.68 (m, 1 H), 3.84 (dd, 2 H, *J* = 0.9, 4.8 Hz), 3.40 (t, 2 H, *J* = 6.1 Hz), 2.43 (s, 3 H), 2.17-2.21 (m, 2 H), 1.81 (quint, 2 H, *J* = 6.0 Hz).

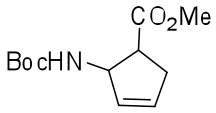
(S)-3-1H-Azepine-1,3-dicarboxylic acid, 2,3,4,7-tetrahydro-, 3-methyl 1-(phenyl methyl) ester (5.41).



5.41

Ring closing metathesis in aqueous media: The diene **5.40** (31 mg) was subjected to RCM (General procedure C6) to give **5.41** as a mixture of rotamers as a colourless oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ H 7.29–7.36 (m, 5H), 5.62–5.79 (m, 2H), 5.09–5.19 (m, 2H), 4.17–4.26 (m, 1H), 3.84 - 4.18 (m, 1H), 3.61-3.74 (m, 4H), 2.91-2.98 (m, 1H), 2.44–2.48 (m, 2H). HRMS (ES) 290.1404 (MH^+); $C_{16}H_{20}NO_4$ requires: 290.1392.

2-*tert*-Butoxycarbonylamino-cyclopent-3-enecarboxylic acid methyl ester (5.43)



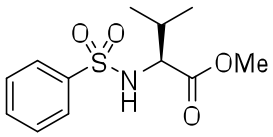
5.43

The diene **5.42** (31 mg) was subjected to RCM (General procedure C6) to give **5.43**. Conversion to **5.43** was determined by analysis of the ^{1}H NMR spectrum of the crude product. Data for **5.43** (from mixture): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.76 (m, 2H), 5.41 (app d, 1H), 4.38 (m, 1H), 3.64 (s, 3H), 2.67 (m, 1H), 2.42 (m, 2H), 1.43 (s, 9H); MS (ES) 242.13 (MH^+).

Lit cit: Gardiner, J.; Anderson, K. H.; Downard, A.; Abell, A. D. *J. Org. Chem.* **2004**, *69*, 3375–3382.

7.6: Experimental work described in chapter 6

2-[(Phenylsulfonyl)amino]-3-methyl butyric acid methyl ester 6.4.

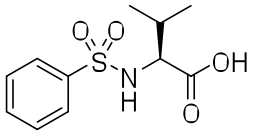


6.4

Benzenesulfonyl chloride **6.3** (500 mg, 2.8 mmol) and valine-OMe **6.2** (470 mg, 1 equiv) were suspended in dried dichloromethane (10 ml) under a nitrogen atmosphere. DIPEA (1.1 ml, 2 equiv) was added dropwise and the mixture stirred overnight. The reaction

solution was diluted by ethyl acetate, washed by brine, dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel and the product eluted with EtOAc/Petroleum ether (1:3) to give the **6.4** (622 mg, 82%) as a white solid. M.p.80-82°C. ^{1}H NMR ($CDCl_3$, 500 MHz): 7.84 (2H, m, Ar*H*), 7.56 (1H, m, Ar*H*), 7.49 (2H, m, Ar*H*), 5.12 (1H, d, *J* = 10.1 Hz, N*H*), 3.75 (1H, dd, *J* = 5.1 and 10.1 Hz, C*H*CH(CH_3)$_2$), 3.42 (3H, s, OCH_3), 2.03 (1H, m, CHC*H*(CH_3)$_2$), 0.95 (3H, d, *J* = 6.8 Hz, CHCH(CH_3)$_2$), 0.87 (3H, d, *J* = 6.8 Hz, CHCH(CH_3)$_2$). ^{13}C NMR ($CDCl_3$, 75 MHz): 171.9, 139.8, 133.0, 129.2, 127.5, 61.3, 52.4, 31.8, 19.2, 17.7. HRMS (ES) 294.0773 (MNa^+); $C_{12}H_{17}NNaO_4S$ requires 294.0770.

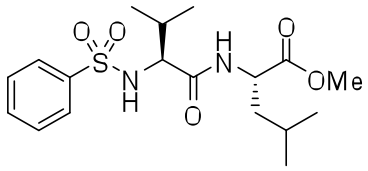
2-Benzenesulfonylamino-3-methyl-butyric acid 6.5.



6.5

To a solution of methyl ether **6.4** (600 mg, 2.2 mmol) in water and THF (1:1, 20ml) was added KOH (4 equiv) and the mixture heated at 40°C for 4 hours, cooled to room temperature and washed with EtOAc (20 ml). The aqueous solution was acidified to pH 1 by the drop wise addition of concentrated HCl. The product was extracted into EtOAc (3x20ml), dried over $MgSO_4$ and concentrated *in vacuo* to give **6.5** as a white solid (537 mg, 95%). Mp. 141-143°C. ^{1}H NMR ($CDCl_3$, 500 MHz): 7.94 (2H, m, Ar*H*), 7.57 (1H, m, Ar*H*), 7.48 (2H, m, Ar*H*), 5.46 (1H, d, *J* = 10.1Hz, N*H*), 3.80 (1H, dd, *J* = 4.7 and 9.9 Hz, C*H*CH(CH_3)$_2$), 2.09 (1H, m, CHC*H*(CH_3)$_2$), 0.96 (3H, d, *J* = 6.8 Hz,CHCH(CH_3)$_2$), 0.87 (3H, d, *J* = 7.0 Hz, CHCH(CH_3)$_2$). ^{13}C NMR ($CDCl_3$, 75 MHz): 176.6, 139.7, 133.2, 129.5, 127.4, 60.9, 31.5, 19.2, 17.3. HRMS (ES) 280.0621 (MNa^+); $C_{11}H_{15}NNaO_4S$ requires 280.0614.

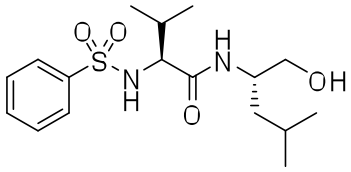
2-(2-Benzenesulfonylamino-3-methyl-butyrylamino)-4-methyl-pentanoic acid methyl ester 6.7.



6.7

To a stirred solution of **6.5** (100 mg) was coupled with Leu-OMe **6.6** (General procedure A1). The crude product was purified by column chromatography on silica gel and elution with EtOAc/Petroleum ether (1:3) to give the **6.7** (130 mg, 87%) as a white solid. m.p 112-114 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): 7.81 (2H, m, Ar*H*), 7.51 (1H, m, Ar*H*), 7.49 (2H, m, Ar*H*), 6.47 (1H, d, *J* = 8.3Hz, N*H*), 5.82 (1H, d, *J* = 8.8Hz, N*H*), 4.32 (1H, m, C*H*$CH_2CH(CH_3)_2$), 3.69 (3H, s, OCH_3), 3.62 (1H, dd, *J* = 5.2 and 8.6Hz, C*H*$CH(CH_3)_2$), 2.01 (1H, m, CHC*H*$(CH_3)_2$), 1.42 (1H, m, CHCH*H*$CH(CH_3)_2$), 1.25-1.29 (2H, m, CHCH*H*$CH(CH_3)_2$ and $CHCH_2CH(CH_3)_2$), 0.88 (3H, d, *J* = 6.8Hz, $CHCH(CH_3)_2$), 0.81 (3H, d, *J* = 6.8Hz, $CHCH(CH_3)_2$), 0.78 (3H, d, *J* = 6.4Hz, $CHCH_2CH(CH_3)_2$), 0.74 (3H, d, *J* = 6.4Hz, $CHCH_2CH$ $(CH_3)_2$). ^{13}C NMR ($CDCl_3$, 75 MHz): 173.1, 170.5, 140.0, 132.8, 129.1, 127.4, 61.7, 52.5, 50.9, 41.2, 32.0, 24.6, 22.8, 21.9, 19.2, 17.3. HRMS (ES) 407.1627 (MNa^+); $C_{18}H_{28}N_2NaO_5S$ requires 407.1611.

2-Benzenesulfonylamino-N-(1-hydroxymethyl-3-methyl-butyl)-3-methyl-butyramide 6.8.

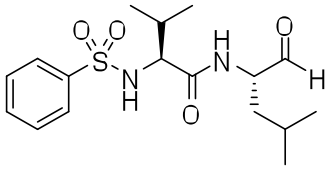


6.8

Methyl ester **6.7** (120 mg, 0.31 mmol) was reduced (General procedure G2) and the crude product was recrystallized from EtOAc to give the **6.8** as a white solid (87 mg, 79%). M.p 152-154 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): 7.86 (2H, m, Ar*H*), 7.61 (1H, m, Ar*H*), 7.52 (2H, m, Ar*H*), 6.12 (1H, d, *J* = 9.0Hz, N*H*), 5.16 (1H, d, *J* = 6.4Hz, N*H*), 3.97 (1H, m, C*H*$CH_2CH(CH_3)_2$), 3.69 (1H, dd, *J* = 3.2 and 10.6Hz, CHC*H*HOH), 3.42-3.46 (2H, C*H*$CH(CH_3)_2$ and CHCH*H*OH), 2.15 (1H, m, CHC*H*$(CH_3)_2$), 1.42 (1H, m, $CHCH_2CH$

$(CH_3)_2$), 1.33 (1H, m, CHC*H*HCH$(CH_3)_2$), 1.31 (1H, m, CHCH*H*CH$(CH_3)_2$), 0.89 (3H, d, *J* = 6.6Hz, $CHCH_2CH(CH_3)_2$), 0.86 (3H, d, *J* = 6.7Hz, CH $CH_2CH(CH_3)_2$). 0.84 (3H, d, *J* = 7.0Hz, $CHCH(CH_3)_2$), 0.78 (3H, d, *J* = 7.0Hz, $CHCH(CH_3)_2$) ^{13}C NMR ($CDCl_3$, 75 MHz): 170.7 (*C*ONH), 139.0, 133.3, 129.4, 127.6, 65.6 , 62.6, 50.1, 41.2, 40.0, 31.3, 24.9, 23.3, 22.3, 19.2, 17.3. HRMS (ES) 379.1679 (MNa^+); $C_{17}H_{28}N_2NaO_4S$ requires 379.1662.

2-Benzenesulfonylamino-N-(1-formyl-3-methyl-butyl)-3-methyl-butyramide 6.9.



6.9

The alcohol **6.8** (50 mg, 0.13 mmol) was oxidized (General procedure H3) and the crude was purified by chromatography on silica gel and elution with ethyl acetate/petroleum ether gave **6.9** as an white solid (36 mg, 72%).^{1}H NMR ($CDCl_3$, 500 MHz): 9.46 (1H, s, CHO), 7.86 (2H, m, Ar*H*), 7.57 (1H, m, Ar*H*), 7.49 (2H, m, Ar*H*), 6.15 (1H, d, *J* = 7.0Hz, N*H*), 5.37 (1H, d, *J* = 8.5Hz, N*H*), 4.38 (1H, m, C*H*$CH_2CH(CH_3)_2$), 3.59 (1H, dd, *J* = 5.2 and 8.6Hz, C*H*$CH(CH_3)_2$), 2.10 (1H, m, CHC*H*$(CH_3)_2$), 1.55 (1H, m, CH CH*H*$CH(CH_3)_2$), 1.41 ($CHCH_2CH(CH_3)_2$), 1.26-1.29 (1H, m, CHC*H*HCH$(CH_3)_2$), 0.84-0.92 (12H, m, $CHCH(CH_3)_2$ and $CHCH_2CH(CH_3)_2$). ^{13}C NMR ($CDCl_3$, 75 MHz): 199.0 (*C*HO), 170.7 (*C*ONH), 133.1 , 129.3, 127.5, 62.0, 57.6, 50.9, 38.0, 31.9, 24.7, 23.2, 22.0, 19.3, 17.2. HRMS (ES) 377.1509 (MNa^+); $C_{17}H_{26}N_2NaO_4S$ requires 377.1505.

References

[1] Abell, A. D.; Jones, M. A.; Coxon, J. M.; Morton, J. D.; Aitken, S. G.; McNabb, S. B.; Lee, H. Y. Y.; Mehrtens, J. M.; Alexander, N. A.; Stuart, B. G.; Neffe, A. T.; Bickerstaffe, R. Molecular *Angew. Chem. Int. Ed.*, **2009**, *48*, 1455 –1458.

Appendix

Appendix 1: Assay of Ovine Calpain 2 Activity

Fluorometric assays (excitation: 485 nm, emission: 520 nm) with ovine calpain 2 were carried out with a (BMG Labtech) Fluostar Optima plate reader at 37.0 ± 0.2 °C in 96-well black (Greiner Bio-one) microassay plates. Calpain 2 partially purified from sheep lung by hydrophobic interaction and ion-exchange chromatography were diluted in 20 mM MOPS, pH 7.5, containing 2 mM EGTA, 2 mM EDTA and 0.035% v/v 2-mercaptoethanol to give a linear response over the course of the assay. The substrate BODIPY-Fl casein was prepared as reported.[1] A 0.0005% solution of the substrate in 10 mM MOPS, pH 7.5, 10 mM $CaCl_2$, 0.1 mM NaN_3, 0.1% v/v 2-mercaptoethanol was prepared freshly before each experiment. Stock solutions of inhibitors (5 mM) were freshly prepared in DMSO and diluted in DMSO/water mixtures to obtain a total DMSO concentration of 4% v/v.

Inhibition studies were performed in the presence of seven different inhibitor concentrations and 1% v/v DMSO in a volume of 200 µL: 50 µl of inhibitor solution was added to a microassay well followed by 50 µl of calpain-containing solution. The reaction was initiated by adding 100 µl of BODIPY-Fl casein solution to each well and progress curves were monitored every 30 s over 570 s. Uninhibited enzyme activity was determined by adding 4% v/v DMSO in water instead of inhibitor solution. Every experiment included two blanks, a Ca^{2+} blank and an EDTA blank. The Ca^{2+} blank contained 50 µl water and 50 µl 20 mM MOPS, pH 7.5, 2 mM EGTA, 2 mM EDTA and 0.035% v/v 2-mercaptoethanol instead of inhibitor and enzyme solution, respectively. For the EDTA blank, 50 µl 50 mM EDTA/NaOH, pH 7.5, was added instead of inhibitor solution to the well.

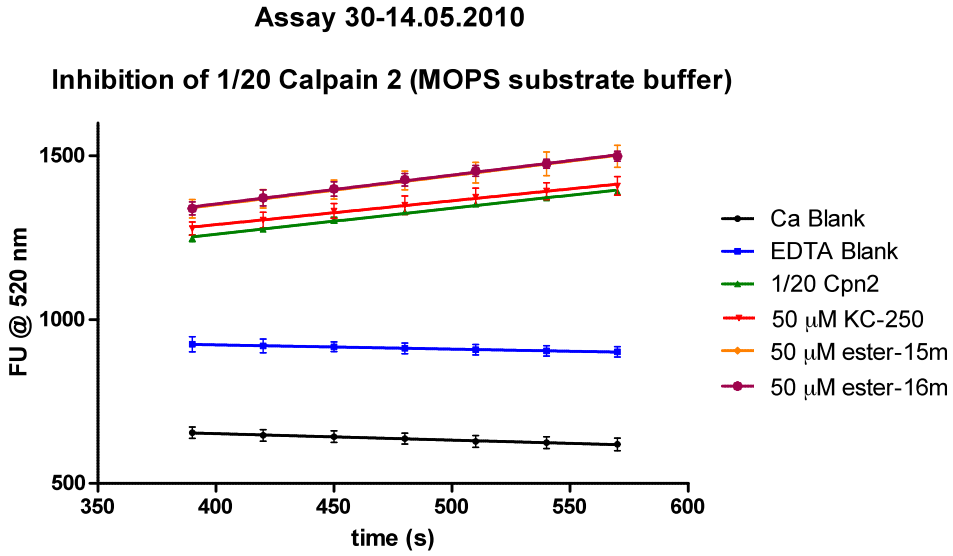
The rate of enzyme-catalyzed substrate hydrolysis was obtained by linear regression of the progress curves over the time course. If slow-binding inhibition occurred[2] only those data points representing the steady state of enzyme-inhibitor interaction were taken into account, i.e. data points between 390 s and 570 s. The rate of the enzymatic reaction was

corrected by the average value of the rates obtained for the two blanks, and the rate in the absence of inhibitor was set to 100%.

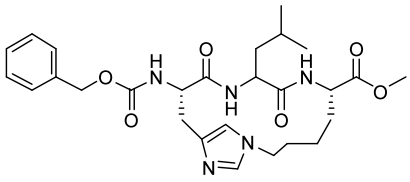
The average value of rates obtained in two separate experiments, each in triplicate, was plotted versus the inhibitor concentration and IC_{50} values were calculated with the following equation:

$$v_i = \frac{v_0}{1 + \frac{[I]}{IC_{50}}}$$

where [I] is the inhibitor concentration, and v_0 and v are the enzyme activities in the absence and presence of inhibitor. All analysis was done with the program GraphPad Prism version 5.02 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com.

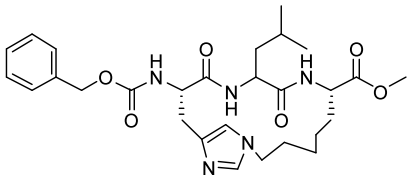


ester-15m



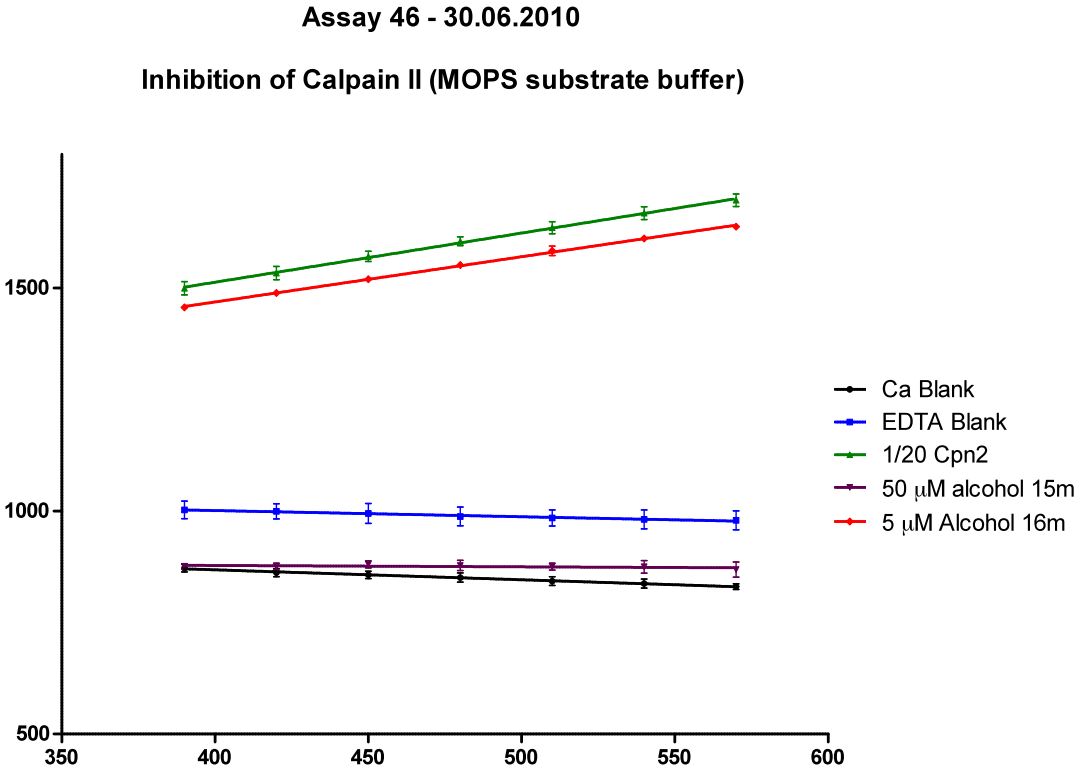
4.1a

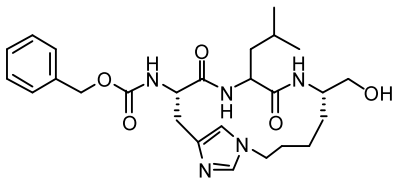
ester-16m



4.2a

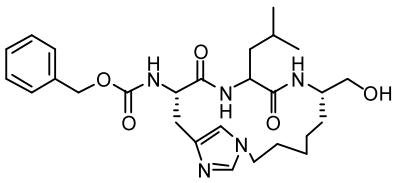
No inhibition of calpain 2 was observed by either esters-15m **4.1a** and ester-16m **4.2a**.





Code: alcohol-15m

4.1b



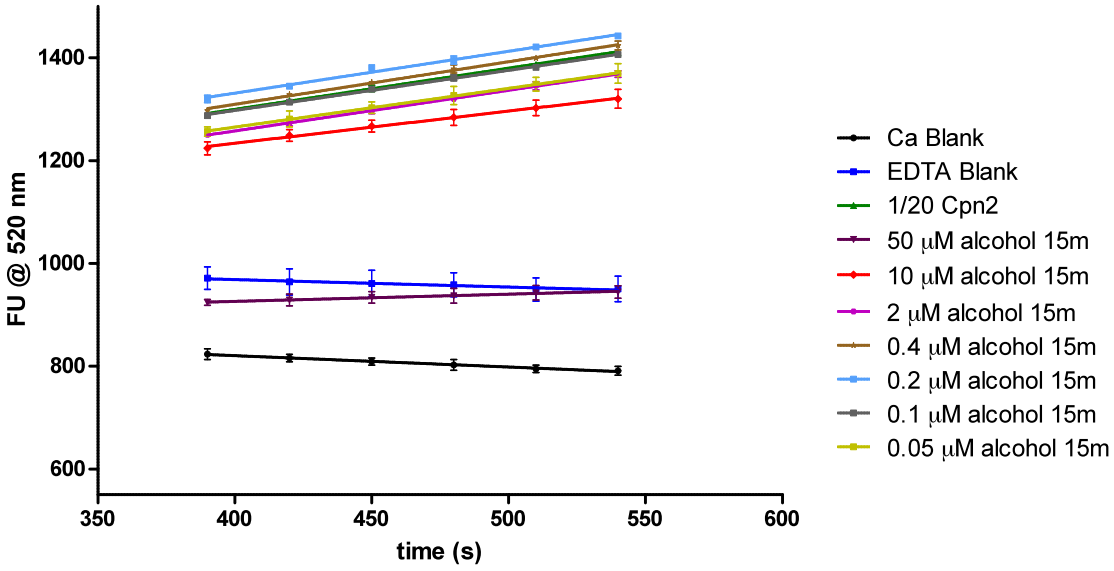
Code: alcohol-16m

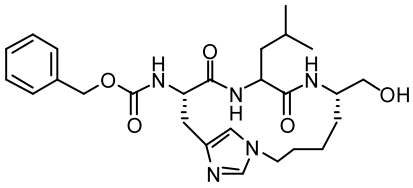
4.2b

No inhibition observed for alcohol-16m **4.2b** at 5µM. Higher concentrations were tried but compound did not dissolve at higher concentrations.

Assay 48 - 30.06.2010

Inhibition of Calpain II (MOPS substrate buffer)



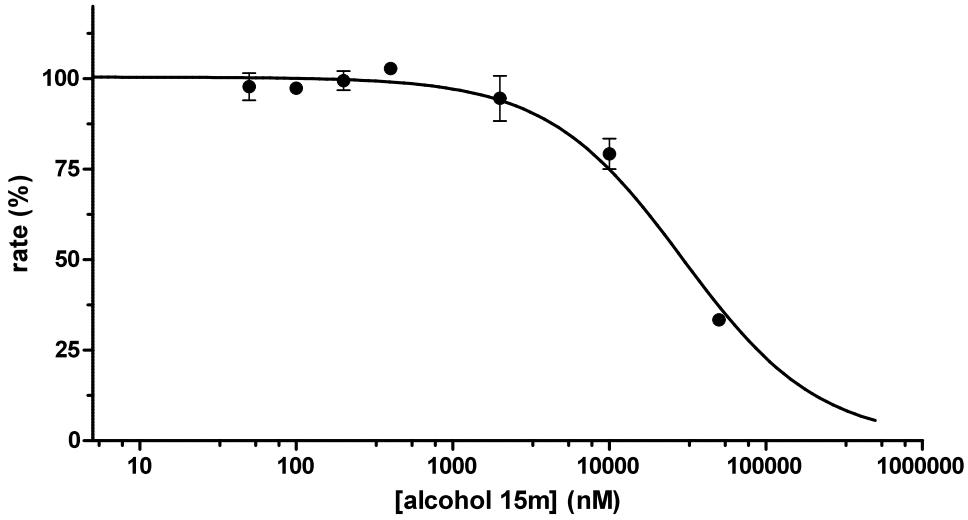


Code: alcohol-15m

4.1b

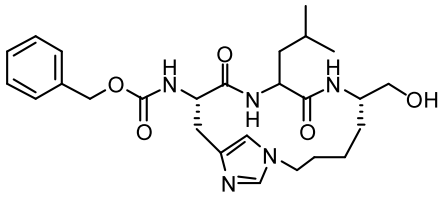
Assay 48 + 49 - 30.06.2010

Inhibition of Calpain II by alcohol 15m (MOPS substrate buffer)
- corrected:
steady-state slope of slow binding inhibition (linear fit: 390-570 s)



IC_{50} = 29.4 ± 2.8 µM

Best-fit values	
v0	100.5
IC50	29390
Std. Error	
v0	1.138
IC50	2773



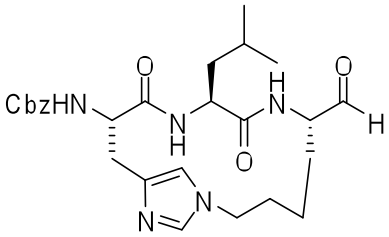
Code: alcohol-15m

4.1b

Compound **4.1b** has an IC_{50} of 29.39 µM.

Assay 102-104 refers to compound 4.1c

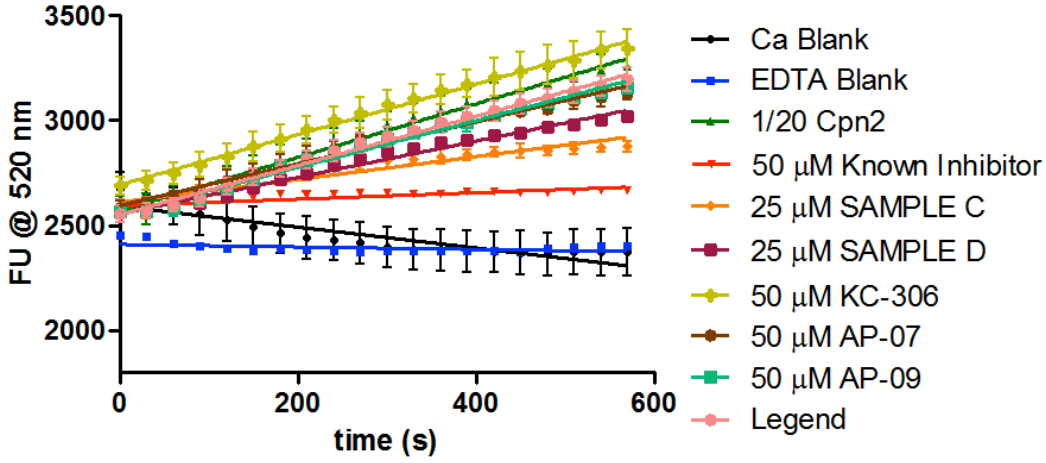
Compound **4.1c** has an IC_{50} of 238nm ± 44nm



4.1c

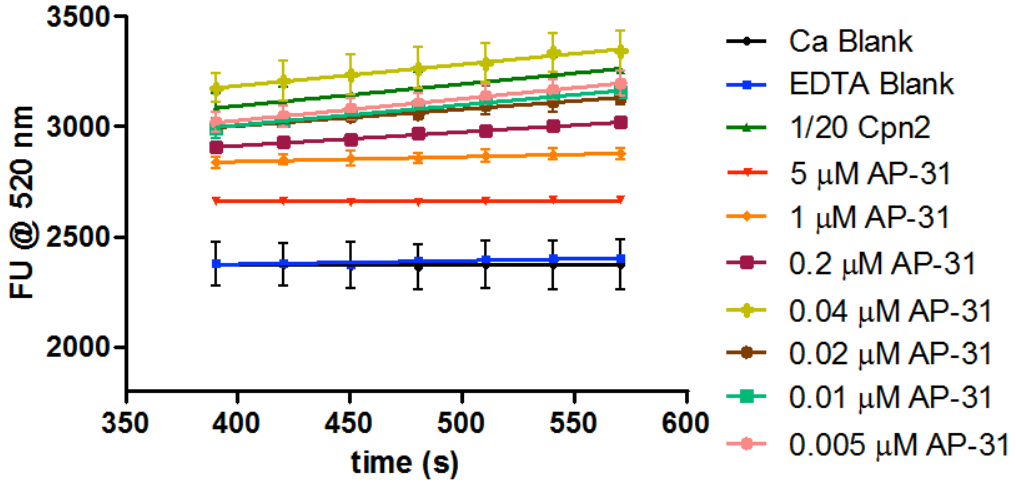
Assay 102 - 07.08.2011

Inhibition of 1/20 Calpain 2 (MOPS substrate buffer)



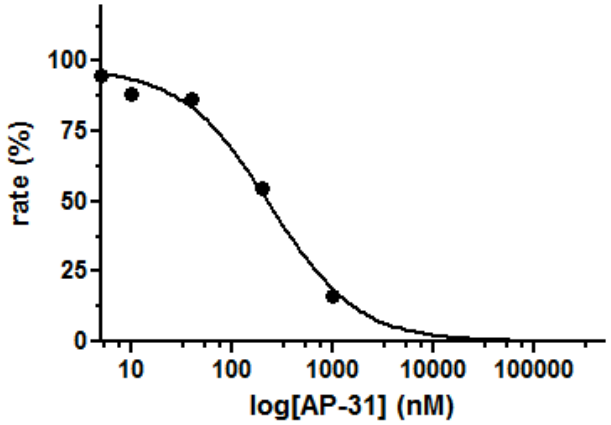
Assay 102 - 07.08.2011

Inhibition of 1/20 Calpain 2 (MOPS substrate buffer)



Average values of Assays 102+104

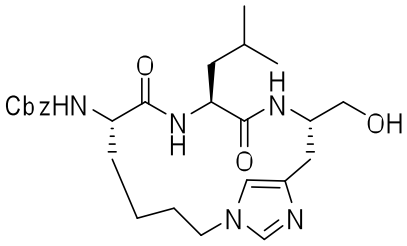
Inhibition of Calpain II by AP-31 (MOPS substrate buffer) - corrected: steady-state slope of slow binding inhibition (linear fit: 390-570 s)



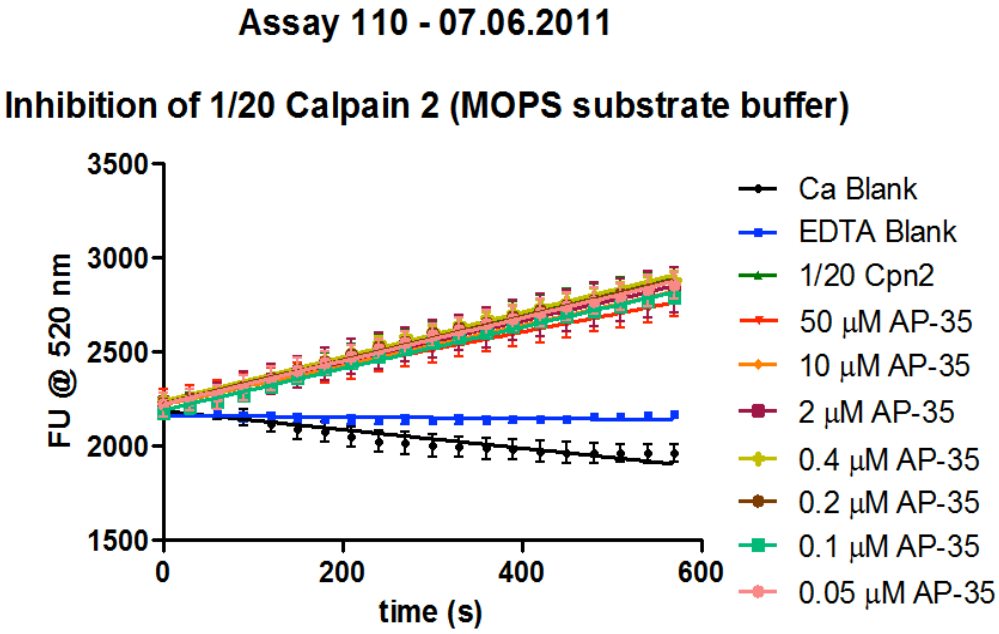
IC_{50} = 238 ± 44 nM

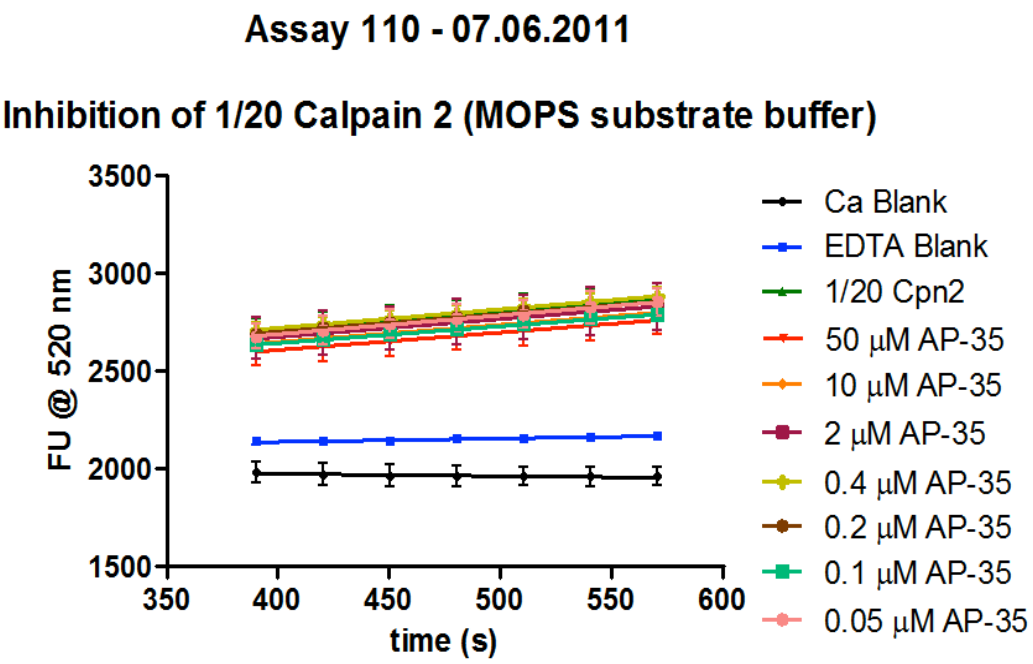
Assay 110-111 refers to compound 4.3b

Compound **4.3b** is inactive with an IC_{50} greater than 50 µm



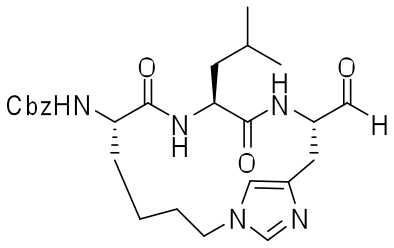
4.3b




Assay 110 - 07.06.2011
Inhibition of 1/20 Calpain 2 (MOPS substrate buffer)
FU @ 520 nm
3500
3000
2500
2000
1500
350
400
450
500
550
600
time (s)
Ca Blank
EDTA Blank
1/20 Cpn2
50 µM AP-35
10 µM AP-35
2 µM AP-35
0.4 µM AP-35
0.2 µM AP-35
0.1 µM AP-35
0.05 µM AP-35

Assay 118-119 refers to compound 4.3c

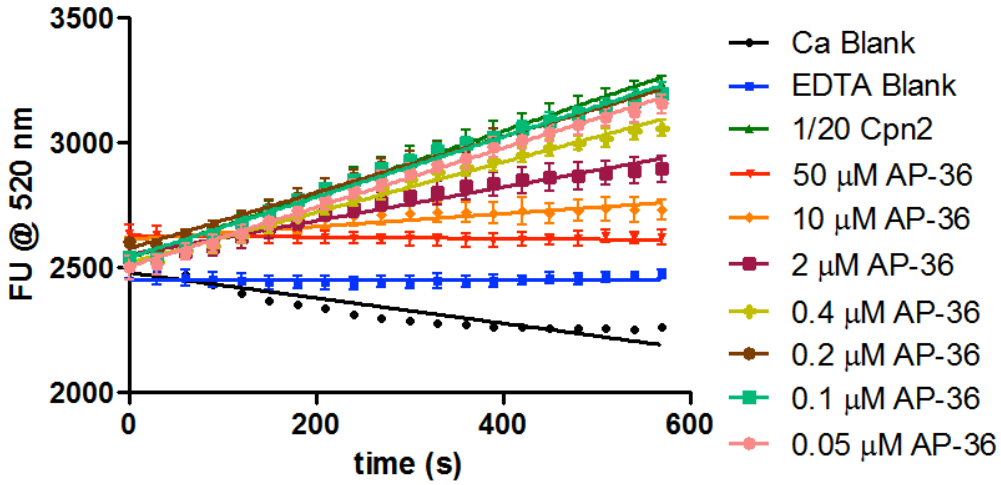
Compound **4.3c** has an IC_{50} of 1 µm ± 0.1 µm.



4.3c

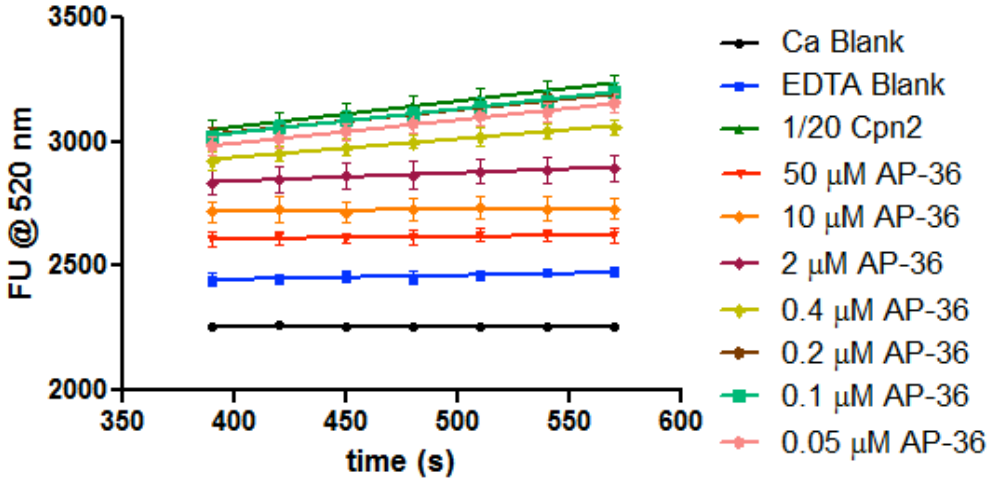
Assay 118 - 09.06.2011

Inhibition of 1/20 Calpain 2 (MOPS substrate buffer)



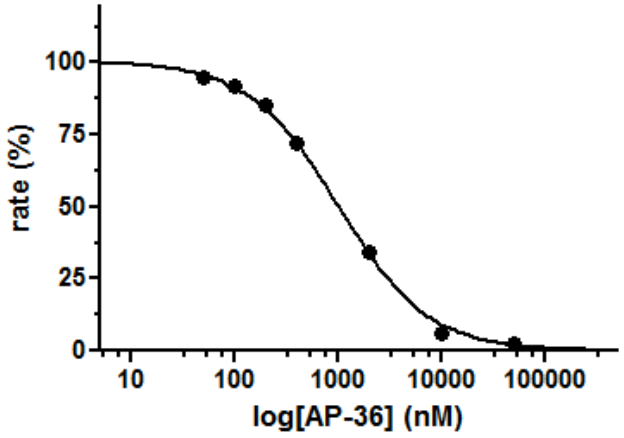
Assay 118 - 09.06.2011

Inhibition of 1/20 Calpain 2 (MOPS substrate buffer)



Average values of Assays 118+119

Inhibition of Calpain II by AP-36 (MOPS substrate buffer)



IC_{50} = 1.0 ± 0.1 µM

Appendix 2: Molecular modeling of histidine containing macrocycles 4.1-4.3

A conformational search has been carried out by Wanting Jiao on the following nine macrocycles **4.1a-c**, **4.2a-c** and **4.3a-c** and docking studies were carried out with a calpain 1 construct model (see Figure A1-A9). The modelling studied showed that:

i. All nine compounds have low energy Glide scores
ii. Macrocyclic alcohols **4.1b** and **4.2b** and aldehydes **4.1c** and **4.2c** form the three essential hydrogen bonds with Gly208 and Gly271 (denoted as A, B and C) that are important for stabilizing a *β*-strand conformation of the peptide chain,
iii. The warhead distance defined as carbonyl carbon to the active cysteine sulfur for macrocyclic aldehydes **4.1c** and **4.2c** is less than 4.5 Å.
iv. Compounds **4.3a-c** do not fit at the P1 pocket of the binding site and poses of **4.3c** do not show it bound in a *β*-strand conformation.

Table A1: Molecular modelling results for histidine-based macrocycles **4.1a-c**, **4.2a-c** and **4.3a-c** studies carried out with Wanting Jiao.

	Glide	Emodel	Essential	WHD [b]	Internal contacts			IC_{50}
compound	Score	Score	H bonds[a]	Å	Good	Bad	Ugly	calpain2
4.1a	-6.05	-65.5	A,C +2	3.51	303	14	3	>50 μM
4.1b	-7.48	-58.5	A,B,C +1	3.69	240	14	2	29 μM
4.1c	-6.05	-64.1	A,B,C +1	3.33	286	13	2	238 nM
4.2a	-4.38	-61.2	A,B +1	4.35	293	13	0	>50μM
4.2b	-6.24	-60.8	A,B,C +3	3.41	245	12	2	
4.2c	-5.72	-59.0	A,B,C +1	3.51	245	13	1	
4.3a	-5.42	-60.2	A,B +1	5.51	253	11	1	>50 μM
4.3b	-6.17	-56.0	A +1	3.79	249	9	2	>50 μM
4.3c	-5.93	-50.8	3	10.7	255	13	0	1 μM

[a] Hydrogen bonds from the carbonyl group of Gly_{208}, the NH group of Gly_{208}, and the carbonyl group of Gly_{271} of the o-CAPN1 homology model are labeled A, B and C respectively. [b] War head distance (WHD) is the distance between the warhead carbon and the active site cysteine sulfur in Å.

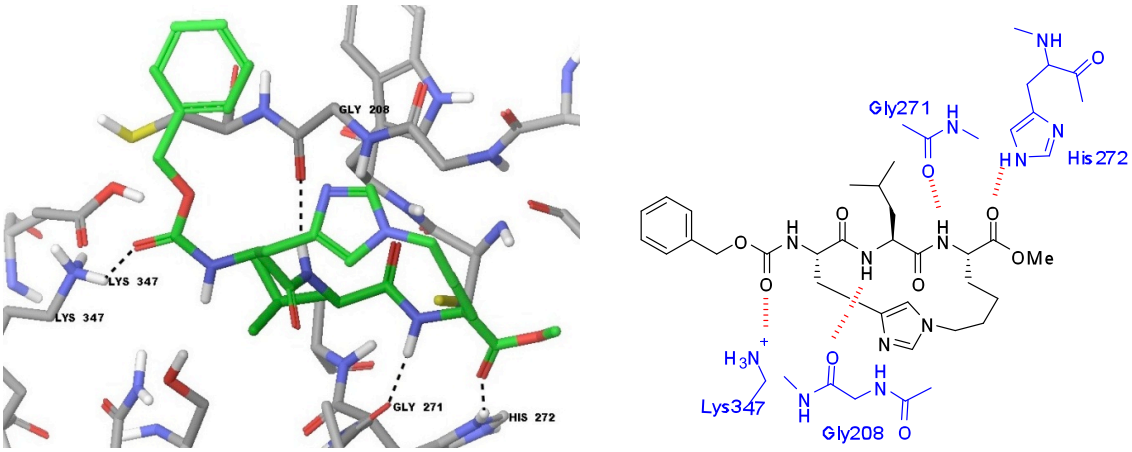


Figure A1: Bonding mode of compound **4.1a**

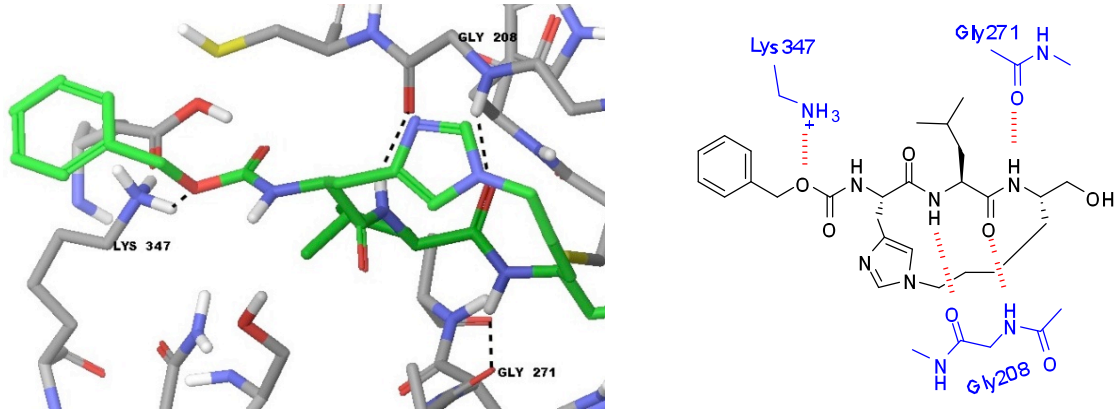


Figure A2: Bonding mode of compound **4.1b**

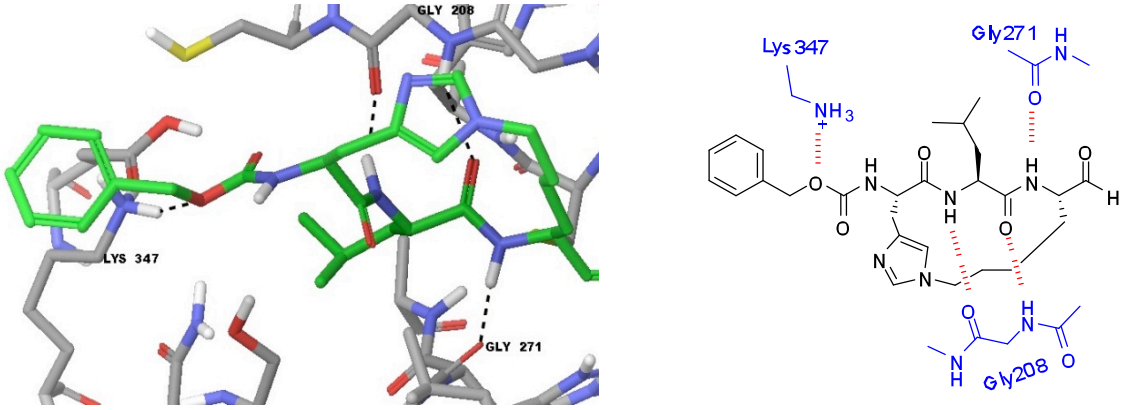


Figure A3: Bonding mode of compound **4.1c**

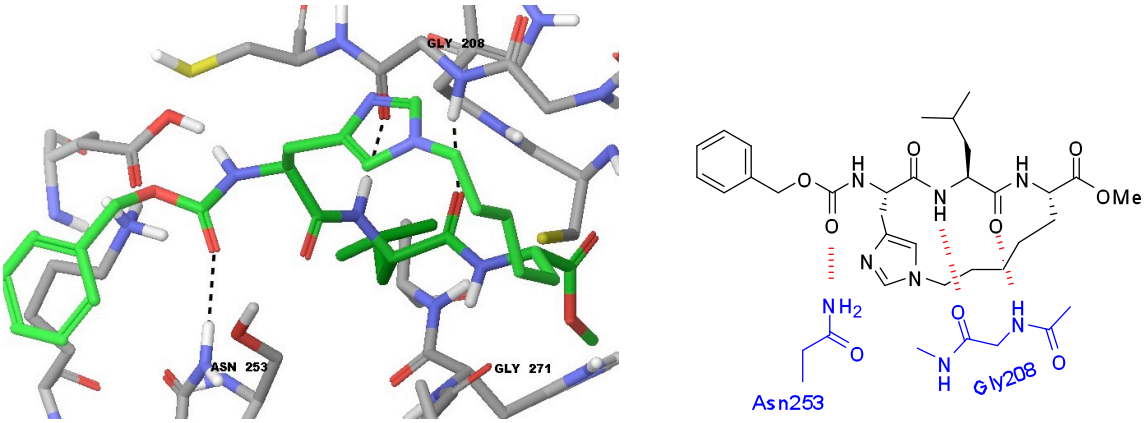


Figure A4: Bonding mode of compound **4.2a**

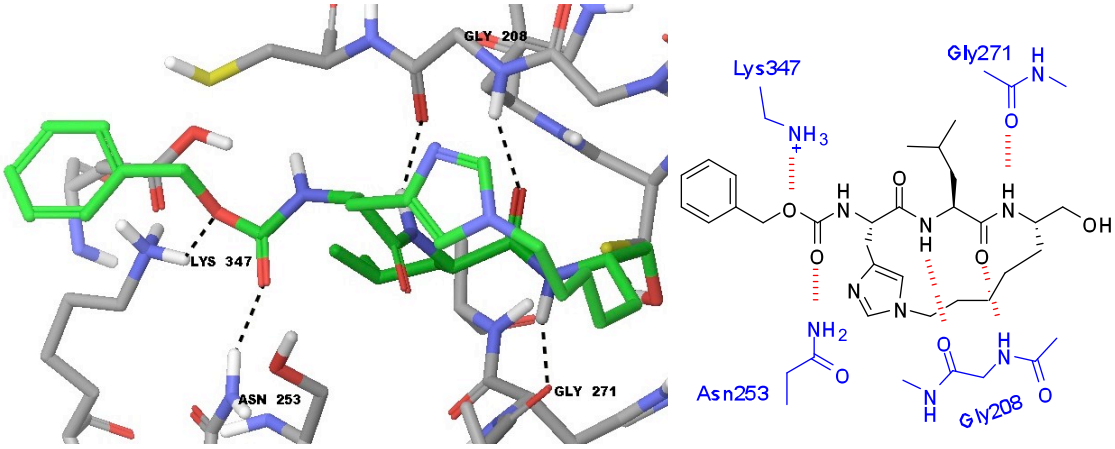


Figure A5: Bonding mode of compound **4.2b**

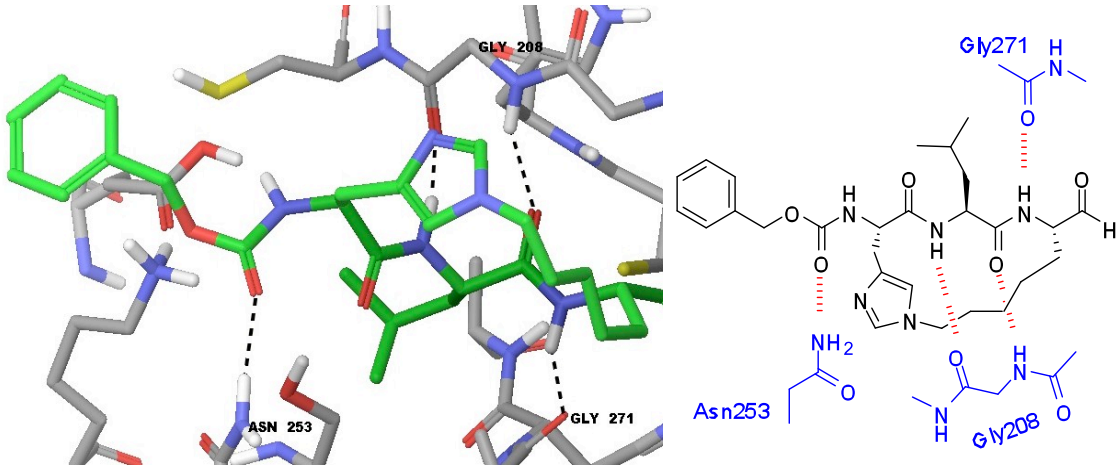


Figure A6: Bonding mode of compound **4.2c**

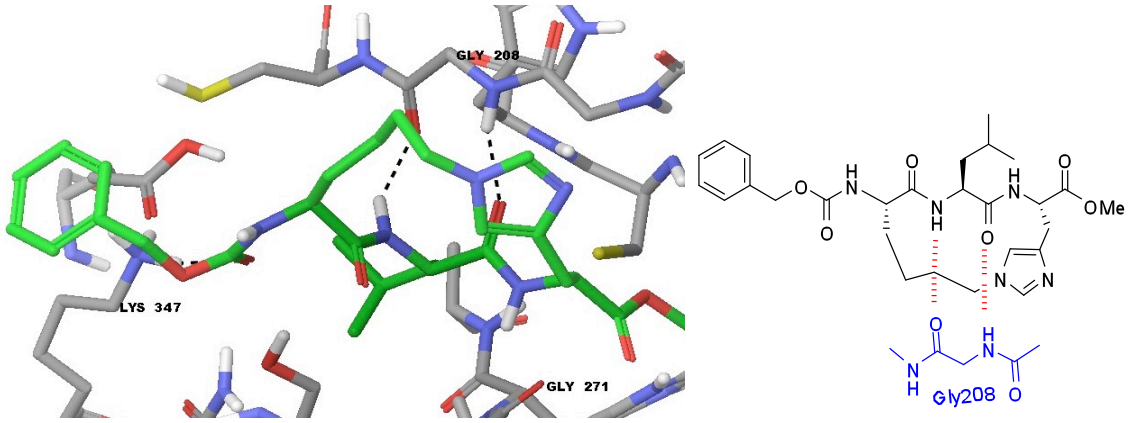


Figure A7: Bonding mode of compound **4.3a**

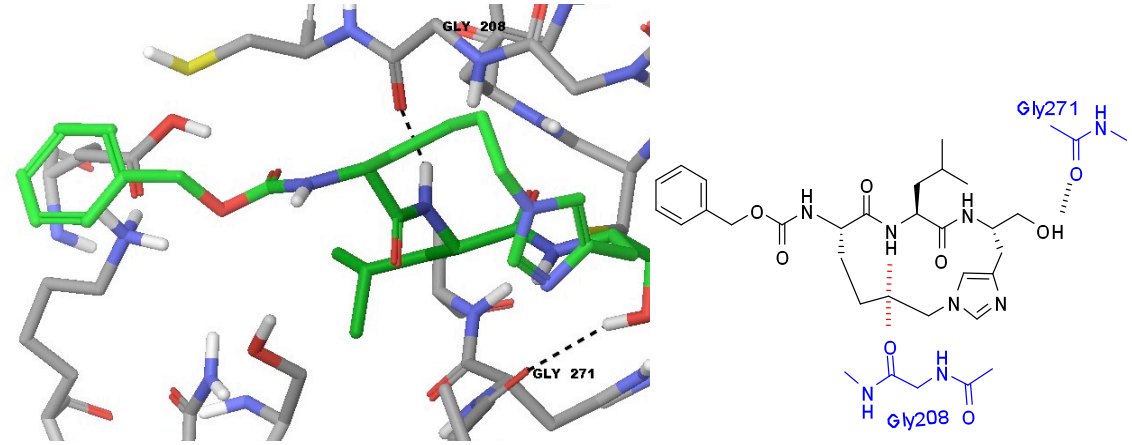


Figure A8: Bonding mode of compound **4.3b**

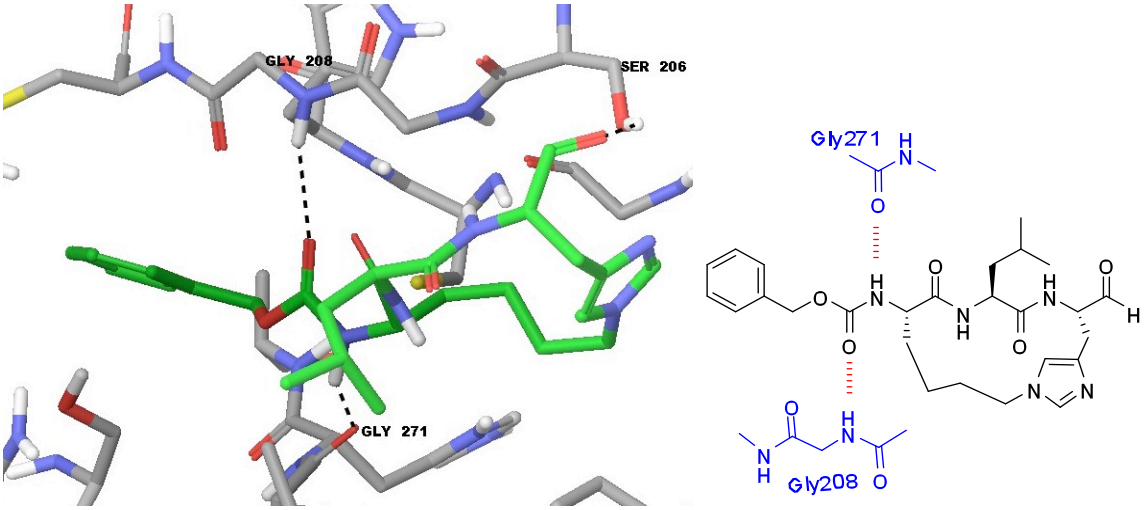


Figure A9: Bonding mode of compound **4.3c**

Appendix 3: X-ray structural analysis of (*E*)-2.2

Analysis on a single crystal was carried out by Dr Matthew Polson at rt. Graphite-monochromated Cu Kα radiation was used for cell parameter determinations and data collection. The intensities of two standard reflections were monitored during data collection to check the stability of the crystals: no loss of intensities was recognized. The integrated intensities, measured using the θ/2θ scan mode, were corrected for Lorentz and polarization effects. The non-hydrogen atoms were refined anisotropically, whereas hydrogen atoms were refined as isotropic. The structures were solved by direct methods of SIR97 and refined using the full-matrix least squares on F^2 provided by SHELXL97.

Aromatic and methyl hydrogens were assigned in calculated positions, the others were found in the Fourier difference synthesis.

Compound **(*E*)-2.2**: $C_{27}H_{39}N_3O_7$, M=517.61, Orthorhombic, space group P 21 21 21, *a*=9.5967(7), *b*=13.576(3), *c*=22.201(6) Å, α=90, *β*=90, γ=90 deg, V=2892.5(11) Å3, Z=4 Dc=1.189, μ=0.086 mm-1, F(000)=1112. The reflections collected were 37305 with a 1.76<θ< 25.10 range; 2923 were independent and the final R index was 0.0729 for reflections having I>2σI, and 0.0529 for all data.

Crystal data and structure refinement for **(*E*)-2.2**.

Table A1. Crystal data and structure refinement for **(*E*)-2.2**.

Identification code	**(*E*)-2.2**
Empirical formula	C27 H39 N3 O7
Formula weight	517.61
Temperature	93(2) K
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	P 21 21 21

Unit cell dimensions	a = 9.5967(17) Å	α= 90°.
	b = 13.576(3) Å	β= 90°.
	c = 22.201(6) Å	ϒ = 90°.
Volume	2892.5(11) $Å^3$	
Z	4	
Density (calculated)	1.189 Mg/m^3	
Absorption coefficient	0.086 mm^{-1}	
F(000)	1112	
Crystal size	0.75 x 0.23 x 0.07 mm^3	
Theta range for data collection	1.76 to 25.10°.	
Index ranges	-11<=h<=11, -16<=k<=16, -26<=l<=26	
Reflections collected	37305	
Independent reflections	2923 [R(int) = 0.0729]	
Completeness to theta = 25.10°	100.0 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	1 and 0.838033	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	2923 / 0 / 349	
Goodness-of-fit on F^2	1.220	
Final R indices [I>2sigma(I)]	R1 = 0.0422, wR2 = 0.0976	
R indices (all data)	R1 = 0.0529, wR2 = 0.1153	
Absolute structure parameter	10(10)	
Extinction coefficient	0.0216(19)	
Largest diff. peak and hole	0.499 and -0.501 e.$Å^{-3}$	

Table A2: Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for **(*E*)-2.2**. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
C(1)	4529(2)	1344(2)	87(1)	19(1)
C(2)	5195(2)	2355(2)	-10(1)	18(1)
C(3)	4854(3)	4129(2)	-90(1)	18(1)
C(4)	4071(2)	4849(2)	308(1)	18(1)
C(5)	4211(3)	6201(2)	1020(1)	25(1)
C(6)	4607(3)	7203(2)	760(1)	28(1)
C(7)	4551(3)	6213(2)	1695(1)	29(1)
C(8)	4195(3)	5284(2)	2020(1)	29(1)
C(9)	4804(3)	5020(2)	2531(1)	31(1)
C(10)	4481(4)	4136(2)	2904(1)	37(1)
C(11)	3950(3)	2912(2)	2157(1)	29(1)
C(12)	2909(3)	2405(2)	1852(1)	30(1)
C(13)	3248(3)	1774(2)	1386(1)	28(1)
C(14)	4620(3)	1627(2)	1215(1)	23(1)
C(15)	5643(3)	2161(2)	1513(1)	27(1)
C(16)	5320(3)	2803(2)	1979(1)	30(1)
C(17)	4976(3)	937(2)	704(1)	23(1)
C(18)	4578(3)	4375(2)	-757(1)	23(1)
C(19)	4917(3)	5420(2)	-961(1)	29(1)
C(20)	4492(4)	5533(3)	-1620(1)	44(1)
C(21)	6430(3)	5691(2)	-877(2)	38(1)
C(22)	4121(3)	131(2)	-717(1)	18(1)
C(23)	4211(3)	-1031(2)	-1564(1)	27(1)
C(24)	3391(3)	-1857(2)	-1276(1)	31(1)
C(25)	5458(3)	-1438(3)	-1908(1)	36(1)
C(26)	3330(3)	-374(2)	-1969(1)	34(1)
C(27)	6037(5)	8083(2)	103(2)	53(1)
N(21)	4985(2)	697(2)	-394(1)	22(1)

N(2)	4352(2)	3130(2)	29(1)	18(1)
N(4)	4834(2)	5385(2)	694(1)	22(1)
O(2)	6456(2)	2427(1)	-112(1)	24(1)
O(4)	2803(2)	4950(2)	252(1)	27(1)
O(6)	4143(3)	7958(2)	957(1)	49(1)
O(10)	3493(2)	3491(2)	2624(1)	38(1)
O(22)	2855(2)	122(2)	-674(1)	26(1)
O(23)	4886(2)	-431(1)	-1097(1)	23(1)
O(27)	5530(2)	7152(2)	321(1)	36(1)

Table A3: Bond lengths [Å] and angles [°] for **(*E*)-2.2**.

C(1)-N(21)	1.449(3)
C(1)-C(2)	1.529(3)
C(1)-C(17)	1.538(4)
C(1)-H(1)	0.9800
C(2)-O(2)	1.234(3)
C(2)-N(2)	1.331(3)
C(3)-N(2)	1.463(3)
C(3)-C(4)	1.517(4)
C(3)-C(18)	1.539(4)
C(3)-H(3)	0.95(3)
C(4)-O(4)	1.231(3)
C(4)-N(4)	1.341(3)
C(5)-N(4)	1.453(3)
C(5)-C(6)	1.525(4)
C(5)-C(7)	1.533(4)
C(5)-H(5)	0.9800
C(6)-O(6)	1.199(4)
C(6)-O(27)	1.319(4)

C(7)-C(8)	1.493(4)
C(7)-H(7A)	0.9700
C(7)-H(7B)	0.9700
C(8)-C(9)	1.325(4)
C(8)-H(8)	0.9300
C(9)-C(10)	1.490(4)
C(9)-H(9)	0.9300
C(10)-O(10)	1.433(4)
C(10)-H(10A)	0.9700
C(10)-H(10B)	0.9700
C(11)-O(10)	1.372(3)
C(11)-C(16)	1.382(4)
C(11)-C(12)	1.389(4)
C(12)-C(13)	1.382(4)
C(12)-H(12)	0.9300
C(13)-C(14)	1.384(4)
C(13)-H(13)	0.9300
C(14)-C(15)	1.389(4)
C(14)-C(17)	1.511(4)
C(15)-C(16)	1.386(4)
C(15)-H(15)	0.9300
C(16)-H(16)	0.9300
C(17)-H(17A)	0.9700
C(17)-H(17B)	0.9700
C(18)-C(19)	1.526(4)
C(18)-H(18A)	0.9700
C(18)-H(18B)	0.9700
C(19)-C(21)	1.509(4)
C(19)-C(20)	1.527(4)
C(19)-H(19)	0.9800
C(20)-H(20A)	0.9600

C(20)-H(20B)	0.9600
C(20)-H(20C)	0.9600
C(21)-H(21A)	0.9600
C(21)-H(21B)	0.9600
C(21)-H(21C)	0.9600
C(22)-O(22)	1.219(3)
C(22)-N(21)	1.340(3)
C(22)-O(23)	1.353(3)
C(23)-O(23)	1.470(3)
C(23)-C(24)	1.513(4)
C(23)-C(26)	1.523(4)
C(23)-C(25)	1.523(4)
C(24)-H(24A)	0.9600
C(24)-H(24B)	0.9600
C(24)-H(24C)	0.9600
C(25)-H(25A)	0.9600
C(25)-H(25B)	0.9600
C(25)-H(25C)	0.9600
C(26)-H(26A)	0.9600
C(26)-H(26B)	0.9600
C(26)-H(26C)	0.9600
C(27)-O(27)	1.438(4)
C(27)-H(27A)	0.9600
C(27)-H(27B)	0.9600
C(27)-H(27C)	0.9600
N(21)-H(21)	0.85(3)
N(2)-H(2)	0.8600
N(4)-H(4)	0.8600

N(21)-C(1)-C(2)	108.2(2)
N(21)-C(1)-C(17)	110.8(2)

C(2)-C(1)-C(17)	109.4(2)
N(21)-C(1)-H(1)	109.5
C(2)-C(1)-H(1)	109.5
C(17)-C(1)-H(1)	109.5
O(2)-C(2)-N(2)	123.0(2)
O(2)-C(2)-C(1)	120.5(2)
N(2)-C(2)-C(1)	116.5(2)
N(2)-C(3)-C(4)	109.2(2)
N(2)-C(3)-C(18)	108.5(2)
C(4)-C(3)-C(18)	109.6(2)
N(2)-C(3)-H(3)	110.6(17)
C(4)-C(3)-H(3)	107.7(16)
C(18)-C(3)-H(3)	111.2(16)
O(4)-C(4)-N(4)	123.0(2)
O(4)-C(4)-C(3)	120.2(2)
N(4)-C(4)-C(3)	116.8(2)
N(4)-C(5)-C(6)	112.9(2)
N(4)-C(5)-C(7)	114.0(2)
C(6)-C(5)-C(7)	107.9(2)
N(4)-C(5)-H(5)	107.2
C(6)-C(5)-H(5)	107.2
C(7)-C(5)-H(5)	107.2
O(6)-C(6)-O(27)	124.3(3)
O(6)-C(6)-C(5)	122.1(3)
O(27)-C(6)-C(5)	113.6(2)
C(8)-C(7)-C(5)	114.5(2)
C(8)-C(7)-H(7A)	108.6
C(5)-C(7)-H(7A)	108.6
C(8)-C(7)-H(7B)	108.6
C(5)-C(7)-H(7B)	108.6
H(7A)-C(7)-H(7B)	107.6

C(9)-C(8)-C(7)	122.8(3)
C(9)-C(8)-H(8)	118.6
C(7)-C(8)-H(8)	118.6
C(8)-C(9)-C(10)	127.0(3)
C(8)-C(9)-H(9)	116.5
C(10)-C(9)-H(9)	116.5
O(10)-C(10)-C(9)	112.9(2)
O(10)-C(10)-H(10A)	109.0
C(9)-C(10)-H(10A)	109.0
O(10)-C(10)-H(10B)	109.0
C(9)-C(10)-H(10B)	109.0
H(10A)-C(10)-H(10B)	107.8
O(10)-C(11)-C(16)	125.6(3)
O(10)-C(11)-C(12)	115.0(3)
C(16)-C(11)-C(12)	119.4(3)
C(13)-C(12)-C(11)	120.2(3)
C(13)-C(12)-H(12)	119.9
C(11)-C(12)-H(12)	119.9
C(12)-C(13)-C(14)	121.3(3)
C(12)-C(13)-H(13)	119.4
C(14)-C(13)-H(13)	119.4
C(13)-C(14)-C(15)	117.7(3)
C(13)-C(14)-C(17)	120.8(2)
C(15)-C(14)-C(17)	121.5(2)
C(16)-C(15)-C(14)	121.7(3)
C(16)-C(15)-H(15)	119.1
C(14)-C(15)-H(15)	119.1
C(11)-C(16)-C(15)	119.6(3)
C(11)-C(16)-H(16)	120.2
C(15)-C(16)-H(16)	120.2
C(14)-C(17)-C(1)	112.5(2)

C(14)-C(17)-H(17A)	109.1
C(1)-C(17)-H(17A)	109.1
C(14)-C(17)-H(17B)	109.1
C(1)-C(17)-H(17B)	109.1
H(17A)-C(17)-H(17B)	107.8
C(19)-C(18)-C(3)	116.8(2)
C(19)-C(18)-H(18A)	108.1
C(3)-C(18)-H(18A)	108.1
C(19)-C(18)-H(18B)	108.1
C(3)-C(18)-H(18B)	108.1
H(18A)-C(18)-H(18B)	107.3
C(21)-C(19)-C(18)	113.3(2)
C(21)-C(19)-C(20)	110.5(3)
C(18)-C(19)-C(20)	108.7(3)
C(21)-C(19)-H(19)	108.1
C(18)-C(19)-H(19)	108.1
C(20)-C(19)-H(19)	108.1
C(19)-C(20)-H(20A)	109.5
C(19)-C(20)-H(20B)	109.5
H(20A)-C(20)-H(20B)	109.5
C(19)-C(20)-H(20C)	109.5
H(20A)-C(20)-H(20C)	109.5
H(20B)-C(20)-H(20C)	109.5
C(19)-C(21)-H(21A)	109.5
C(19)-C(21)-H(21B)	109.5
H(21A)-C(21)-H(21B)	109.5
C(19)-C(21)-H(21C)	109.5
H(21A)-C(21)-H(21C)	109.5
H(21B)-C(21)-H(21C)	109.5
O(22)-C(22)-N(21)	125.5(2)
O(22)-C(22)-O(23)	125.8(2)

N(21)-C(22)-O(23)	108.8(2)
O(23)-C(23)-C(24)	109.9(2)
O(23)-C(23)-C(26)	109.7(2)
C(24)-C(23)-C(26)	113.3(2)
O(23)-C(23)-C(25)	102.0(2)
C(24)-C(23)-C(25)	110.6(2)
C(26)-C(23)-C(25)	110.6(2)
C(23)-C(24)-H(24A)	109.5
C(23)-C(24)-H(24B)	109.5
H(24A)-C(24)-H(24B)	109.5
C(23)-C(24)-H(24C)	109.5
H(24A)-C(24)-H(24C)	109.5
H(24B)-C(24)-H(24C)	109.5
C(23)-C(25)-H(25A)	109.5
C(23)-C(25)-H(25B)	109.5
H(25A)-C(25)-H(25B)	109.5
C(23)-C(25)-H(25C)	109.5
H(25A)-C(25)-H(25C)	109.5
H(25B)-C(25)-H(25C)	109.5
C(23)-C(26)-H(26A)	109.5
C(23)-C(26)-H(26B)	109.5
H(26A)-C(26)-H(26B)	109.5
C(23)-C(26)-H(26C)	109.5
H(26A)-C(26)-H(26C)	109.5
H(26B)-C(26)-H(26C)	109.5
O(27)-C(27)-H(27A)	109.5
O(27)-C(27)-H(27B)	109.5
H(27A)-C(27)-H(27B)	109.5
O(27)-C(27)-H(27C)	109.5
H(27A)-C(27)-H(27C)	109.5
H(27B)-C(27)-H(27C)	109.5

C(22)-N(21)-C(1)	123.8(2)
C(22)-N(21)-H(21)	117(2)
C(1)-N(21)-H(21)	118(2)
C(2)-N(2)-C(3)	121.4(2)
C(2)-N(2)-H(2)	119.3
C(3)-N(2)-H(2)	119.3
C(4)-N(4)-C(5)	120.5(2)
C(4)-N(4)-H(4)	119.8
C(5)-N(4)-H(4)	119.8
C(11)-O(10)-C(10)	117.8(2)
C(22)-O(23)-C(23)	120.89(19)
C(6)-O(27)-C(27)	115.5(3)

Symmetry transformations used to generate equivalent atoms:

Table A4: Anisotropic displacement parameters ($Å^2 x\ 10^3$) for **(*E*)-2.2**. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + ...\ + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
C(1)	14(1)	17(1)	24(1)	-4(1)	0(1)	0(1)
C(2)	14(1)	18(1)	20(1)	-2(1)	-2(1)	0(1)
C(3)	13(1)	15(1)	27(1)	0(1)	-1(1)	-1(1)
C(4)	16(1)	16(1)	23(1)	4(1)	1(1)	-2(1)
C(5)	19(1)	26(1)	29(1)	-7(1)	-1(1)	5(1)
C(6)	26(1)	26(2)	31(1)	-7(1)	-9(1)	6(1)
C(7)	26(1)	31(2)	31(1)	-10(1)	1(1)	-1(1)
C(8)	26(1)	33(2)	28(2)	-9(1)	5(1)	-4(1)
C(9)	32(2)	32(2)	28(1)	-10(1)	5(1)	-3(1)
C(10)	48(2)	43(2)	21(1)	-8(1)	5(1)	-5(2)
C(11)	40(2)	27(2)	20(1)	4(1)	7(1)	-5(1)

C(12)	27(1)	35(2)	30(2)	3(1)	8(1)	-2(1)
C(13)	25(1)	28(1)	29(1)	2(1)	-1(1)	-6(1)
C(14)	26(1)	22(1)	22(1)	7(1)	-3(1)	1(1)
C(15)	25(1)	28(1)	29(1)	4(1)	-2(1)	-3(1)
C(16)	35(2)	32(2)	25(1)	0(1)	-3(1)	-8(1)
C(17)	20(1)	19(1)	30(1)	4(1)	-2(1)	0(1)
C(18)	21(1)	21(1)	26(1)	-2(1)	1(1)	3(1)
C(19)	35(2)	23(1)	28(1)	3(1)	5(1)	8(1)
C(20)	60(2)	39(2)	32(2)	9(2)	4(2)	7(2)
C(21)	41(2)	27(2)	47(2)	9(1)	9(2)	-5(1)
C(22)	18(1)	17(1)	20(1)	1(1)	-2(1)	1(1)
C(23)	24(1)	30(2)	27(1)	-11(1)	-3(1)	-2(1)
C(24)	27(1)	26(2)	40(2)	-10(1)	-3(1)	-2(1)
C(25)	31(2)	42(2)	36(2)	-17(2)	4(1)	1(2)
C(26)	32(2)	43(2)	27(2)	-4(1)	-3(1)	-2(2)
C(27)	78(3)	28(2)	51(2)	8(2)	8(2)	-8(2)
N(21)	13(1)	20(1)	32(1)	-9(1)	0(1)	2(1)
N(2)	12(1)	15(1)	27(1)	0(1)	2(1)	-1(1)
N(4)	17(1)	22(1)	25(1)	-3(1)	-2(1)	4(1)
O(2)	13(1)	20(1)	38(1)	-1(1)	2(1)	0(1)
O(4)	15(1)	26(1)	40(1)	-7(1)	2(1)	-1(1)
O(6)	60(2)	28(1)	58(1)	-9(1)	5(1)	15(1)
O(10)	48(1)	39(1)	27(1)	-5(1)	13(1)	-9(1)
O(22)	14(1)	32(1)	32(1)	-8(1)	1(1)	-2(1)
O(23)	18(1)	26(1)	26(1)	-8(1)	-1(1)	-1(1)
O(27)	46(1)	22(1)	38(1)	3(1)	7(1)	2(1)

Table A5: Hydrogen coordinates (x 10^4) and isotropic displacement parameters (Å^{2}x 10^3) for **(*E*)-2.2**.

	x	y	z	U(eq)
H(1)	3512	1407	75	22
H(5)	3198	6136	982	30
H(7A)	5539	6340	1744	35
H(7B)	4050	6753	1881	35
H(8)	3518	4872	1857	35
H(9)	5509	5430	2672	37
H(10A)	5336	3776	2979	45
H(10B)	4115	4350	3289	45
H(12)	1982	2490	1962	36
H(13)	2542	1442	1184	33
H(15)	6568	2086	1398	32
H(16)	6022	3158	2169	36
H(17A)	4520	308	771	27
H(17B)	5974	823	701	27
H(18A)	5115	3921	-1002	27
H(18B)	3601	4251	-839	27
H(19)	4354	5877	-721	34
H(20A)	4981	5057	-1860	65
H(20B)	4720	6184	-1756	65
H(20C)	3506	5428	-1658	65
H(21A)	6570	6365	-994	58
H(21B)	6999	5271	-1123	58
H(21C)	6683	5609	-462	58
H(24A)	2651	-1585	-1037	47
H(24B)	3996	-2240	-1023	47
H(24C)	3004	-2271	-1584	47

H(25A)	6038	-1809	-1639	54
H(25B)	5985	-903	-2075	54
H(25C)	5138	-1858	-2227	54
H(26A)	3878	176	-2103	51
H(26B)	2537	-139	-1749	51
H(26C)	3020	-745	-2312	51
H(27A)	6518	8420	421	79
H(27B)	5266	8477	-31	79
H(27C)	6665	7973	-227	79
H(2)	3494	3043	127	22
H(4)	5698	5244	749	26
H(3)	5810(30)	4180(20)	3(11)	16(7)
H(21)	5850(40)	580(20)	-423(13)	27(8)

Table A6: Torsion angles [°] for **(*E*)-2.2**.

N(21)-C(1)-C(2)-O(2)	-48.6(3)
C(17)-C(1)-C(2)-O(2)	72.1(3)
N(21)-C(1)-C(2)-N(2)	132.2(2)
C(17)-C(1)-C(2)-N(2)	-107.0(2)
N(2)-C(3)-C(4)-O(4)	-63.1(3)
C(18)-C(3)-C(4)-O(4)	55.7(3)
N(2)-C(3)-C(4)-N(4)	119.7(2)
C(18)-C(3)-C(4)-N(4)	-121.5(2)
N(4)-C(5)-C(6)-O(6)	176.8(3)
C(7)-C(5)-C(6)-O(6)	-56.2(4)
N(4)-C(5)-C(6)-O(27)	-5.3(3)
C(7)-C(5)-C(6)-O(27)	121.6(2)
N(4)-C(5)-C(7)-C(8)	-54.8(3)

C(6)-C(5)-C(7)-C(8)	178.9(2)
C(5)-C(7)-C(8)-C(9)	156.7(3)
C(7)-C(8)-C(9)-C(10)	176.9(3)
C(8)-C(9)-C(10)-O(10)	6.3(4)
O(10)-C(11)-C(12)-C(13)	-177.9(2)
C(16)-C(11)-C(12)-C(13)	1.9(4)
C(11)-C(12)-C(13)-C(14)	0.5(4)
C(12)-C(13)-C(14)-C(15)	-2.2(4)
C(12)-C(13)-C(14)-C(17)	-179.9(3)
C(13)-C(14)-C(15)-C(16)	1.7(4)
C(17)-C(14)-C(15)-C(16)	179.3(2)
O(10)-C(11)-C(16)-C(15)	177.4(3)
C(12)-C(11)-C(16)-C(15)	-2.4(4)
C(14)-C(15)-C(16)-C(11)	0.6(4)
C(13)-C(14)-C(17)-C(1)	70.3(3)
C(15)-C(14)-C(17)-C(1)	-107.3(3)
N(21)-C(1)-C(17)-C(14)	175.6(2)
C(2)-C(1)-C(17)-C(14)	56.4(3)
N(2)-C(3)-C(18)-C(19)	175.0(2)
C(4)-C(3)-C(18)-C(19)	55.9(3)
C(3)-C(18)-C(19)-C(21)	60.1(3)
C(3)-C(18)-C(19)-C(20)	-176.6(2)
O(22)-C(22)-N(21)-C(1)	4.1(4)
O(23)-C(22)-N(21)-C(1)	-175.6(2)
C(2)-C(1)-N(21)-C(22)	-131.6(2)
C(17)-C(1)-N(21)-C(22)	108.5(3)
O(2)-C(2)-N(2)-C(3)	4.5(4)
C(1)-C(2)-N(2)-C(3)	-176.4(2)
C(4)-C(3)-N(2)-C(2)	-147.3(2)
C(18)-C(3)-N(2)-C(2)	93.3(3)
O(4)-C(4)-N(4)-C(5)	-7.3(4)

C(3)-C(4)-N(4)-C(5)	169.9(2)
C(6)-C(5)-N(4)-C(4)	-103.1(3)
C(7)-C(5)-N(4)-C(4)	133.3(3)
C(16)-C(11)-O(10)-C(10)	8.6(4)
C(12)-C(11)-O(10)-C(10)	-171.7(2)
C(9)-C(10)-O(10)-C(11)	75.3(3)
O(22)-C(22)-O(23)-C(23)	8.5(4)
N(21)-C(22)-O(23)-C(23)	-171.8(2)
C(24)-C(23)-O(23)-C(22)	-67.9(3)
C(26)-C(23)-O(23)-C(22)	57.3(3)
C(25)-C(23)-O(23)-C(22)	174.6(2)
O(6)-C(6)-O(27)-C(27)	3.7(4)
C(5)-C(6)-O(27)-C(27)	-174.1(3)

Symmetry transformations used to generate equivalent atoms:

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